Structures of photosynthetic pigment-protein complexes and probing electrostatic field inside them by Stark spectroscopy

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Contents

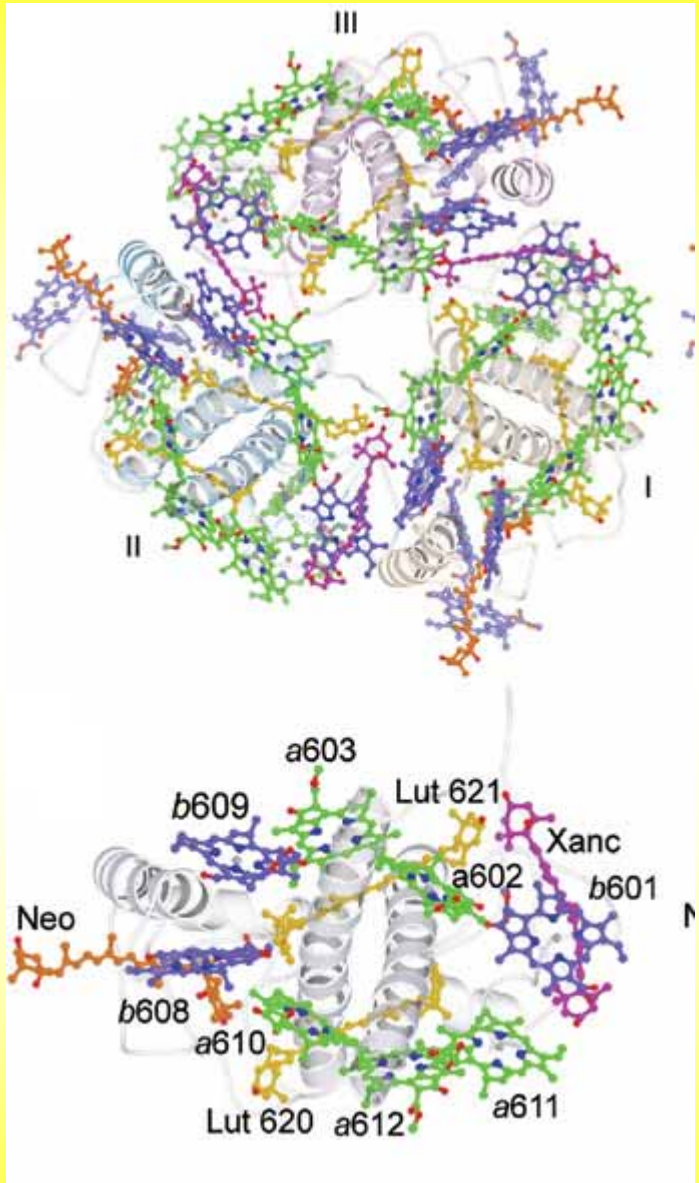
- A comparative look at the structural requirements for photosynthetic light-harvesting
- Structural study of the RC of purple photosynthetic bacteria
- Stark spectroscopy on the native and reconstituted pigment-protein complexes of purple photosynthetic bacteria

- The structure of all photosynthetic reaction centres is strongly conserved.

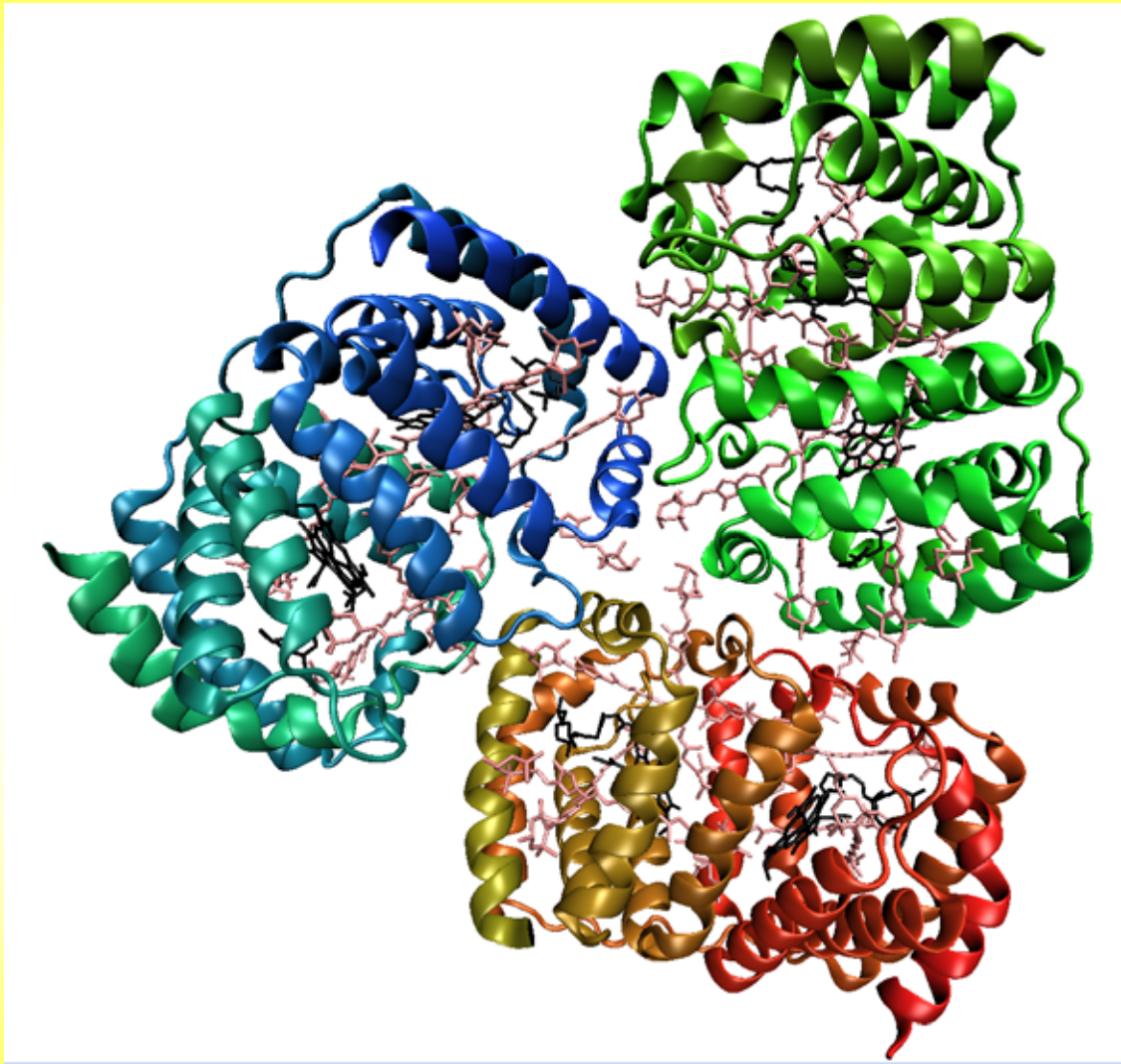
- This is because the structural constraints on electron transfer are very strict.

- A comparison of the structures of photosynthetic light-harvesting complexes shows that they form a very heterogeneous group. Why is this?

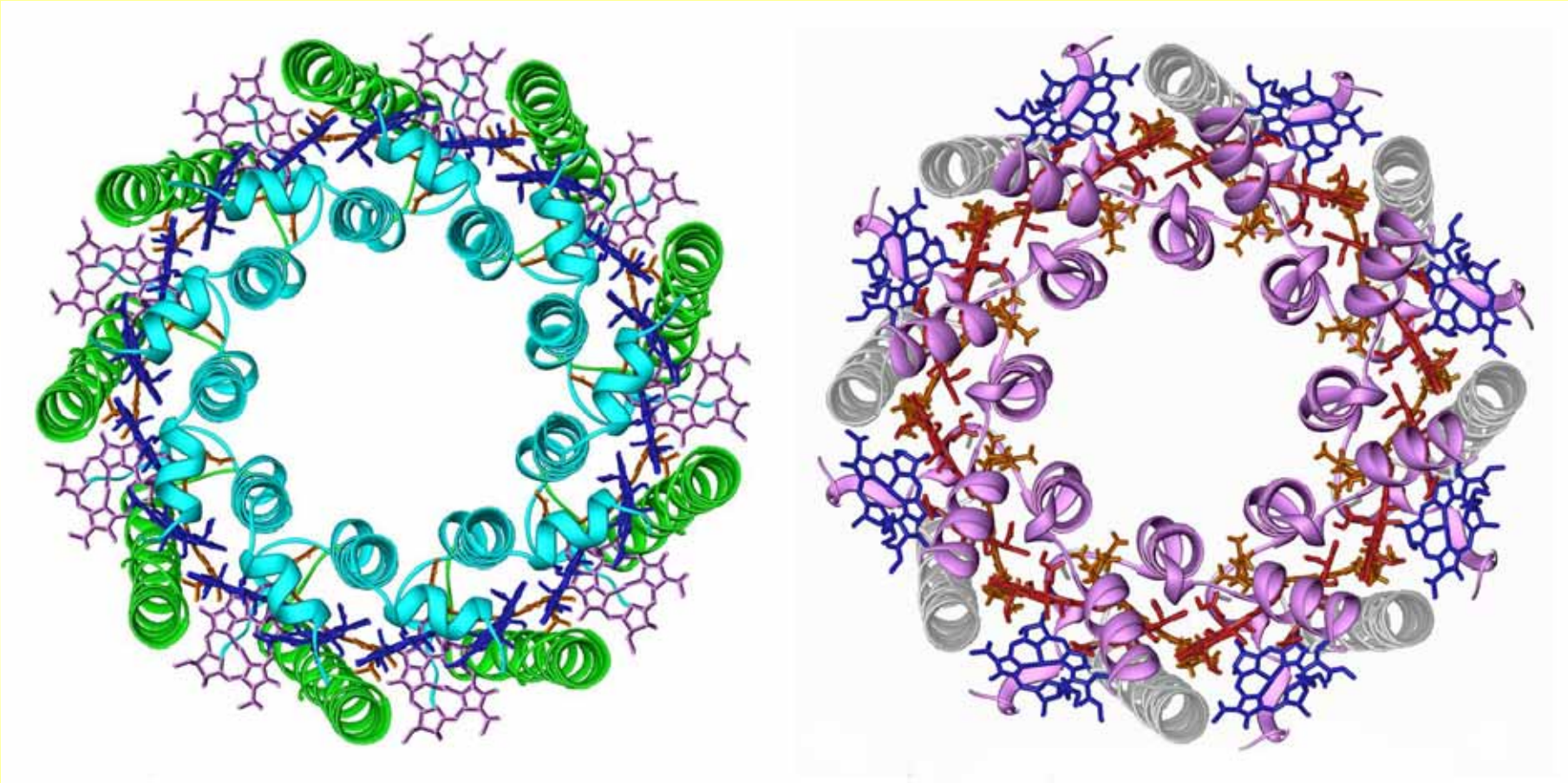
LHCII from spinach



Peridinin-Chlorophyll a complex

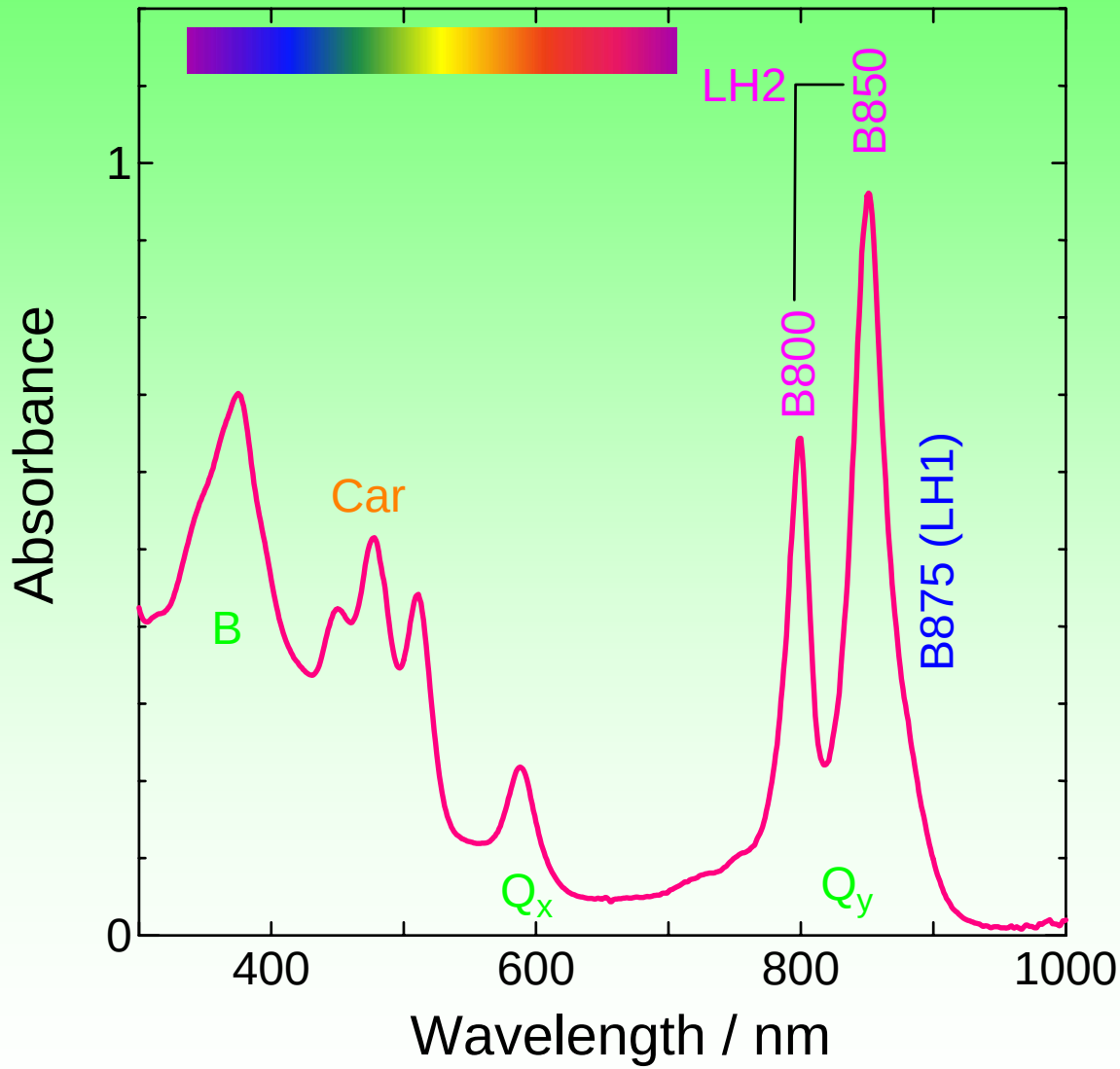


LH2 complexes

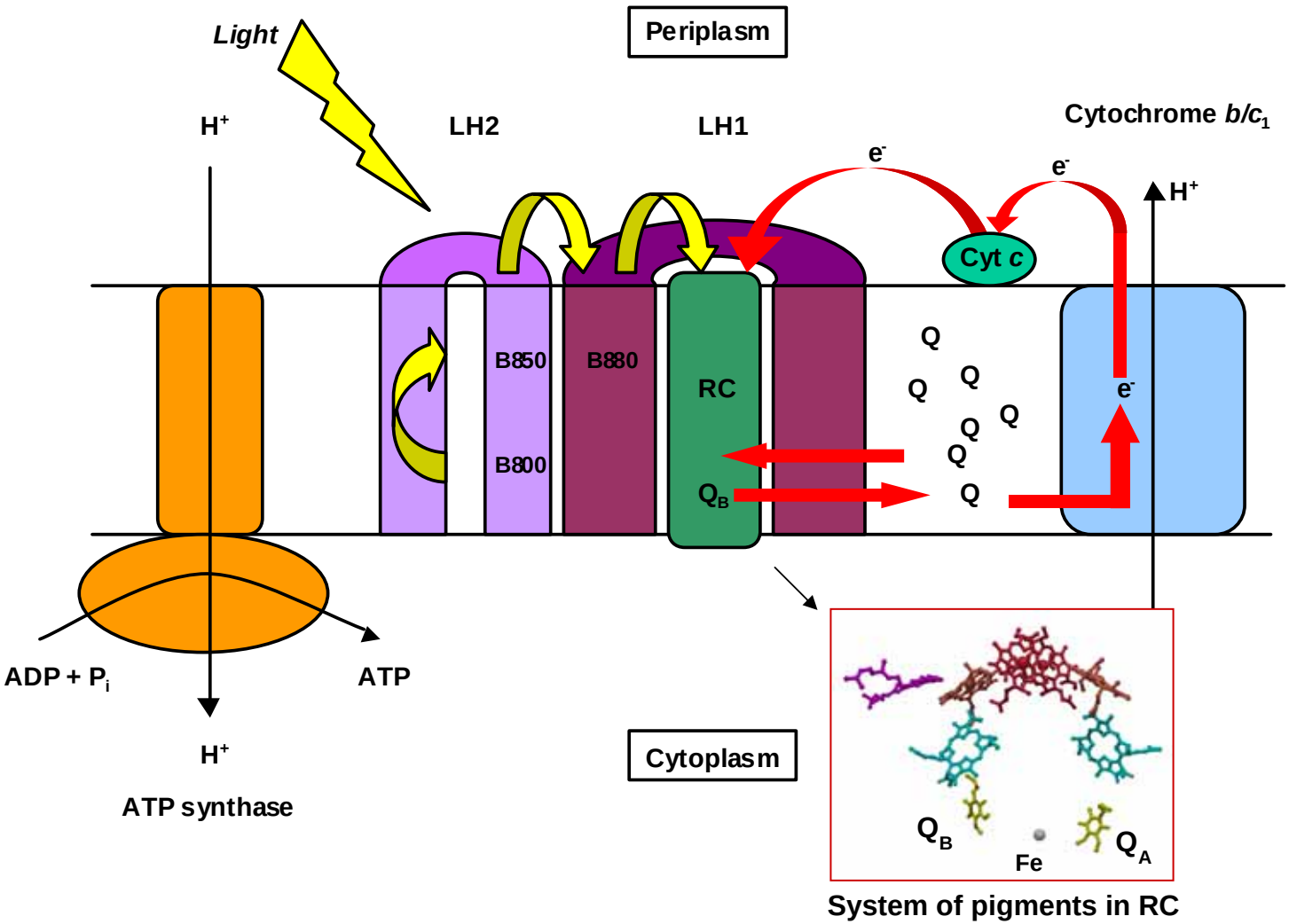


The physics of energy transfer is rather tolerant. This means that there are many structural solutions to the problem of building an efficient light-harvesting complex.

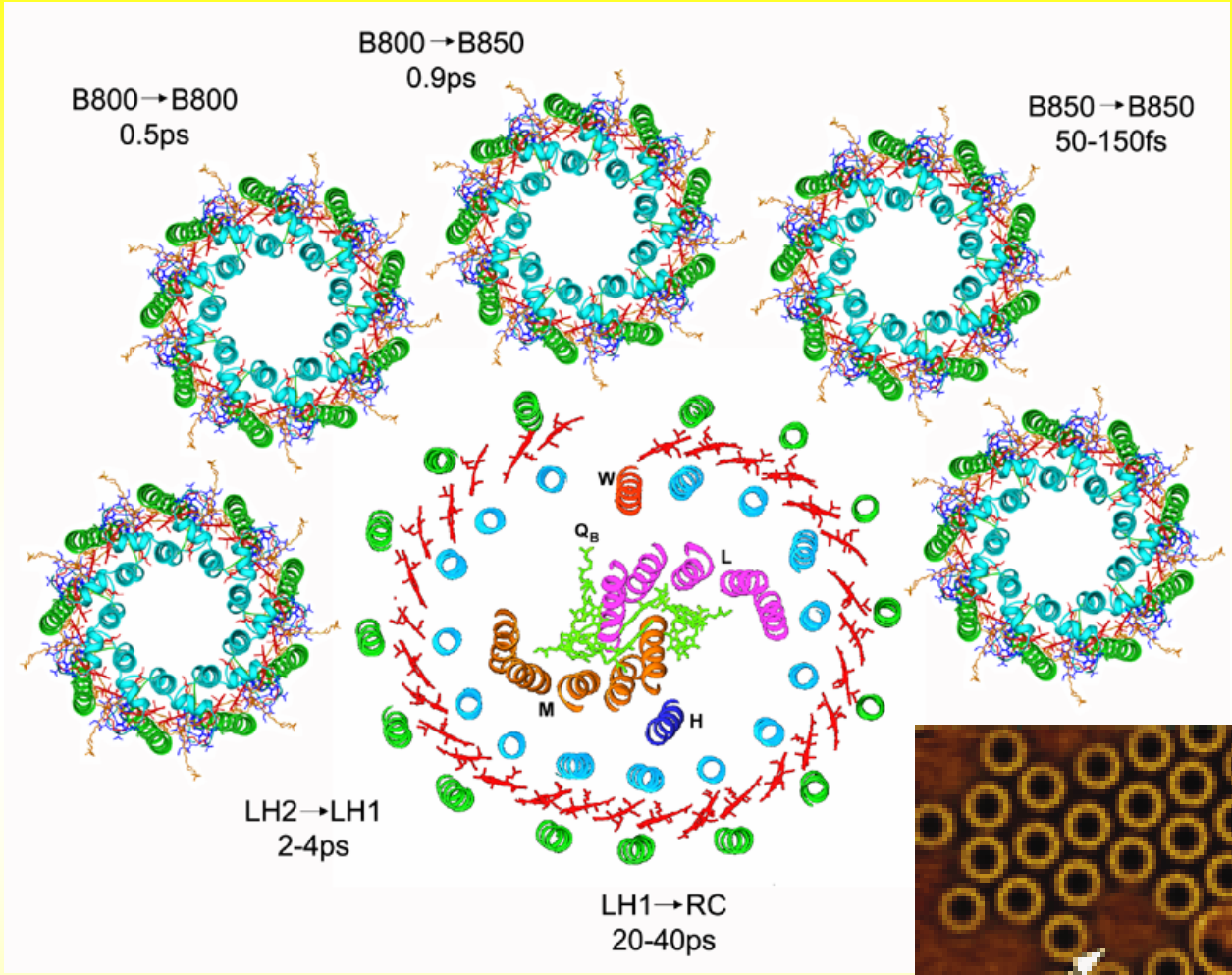
Absorption spectrum of the chromatophores of *Rb. sphaeroides* 2.4.1

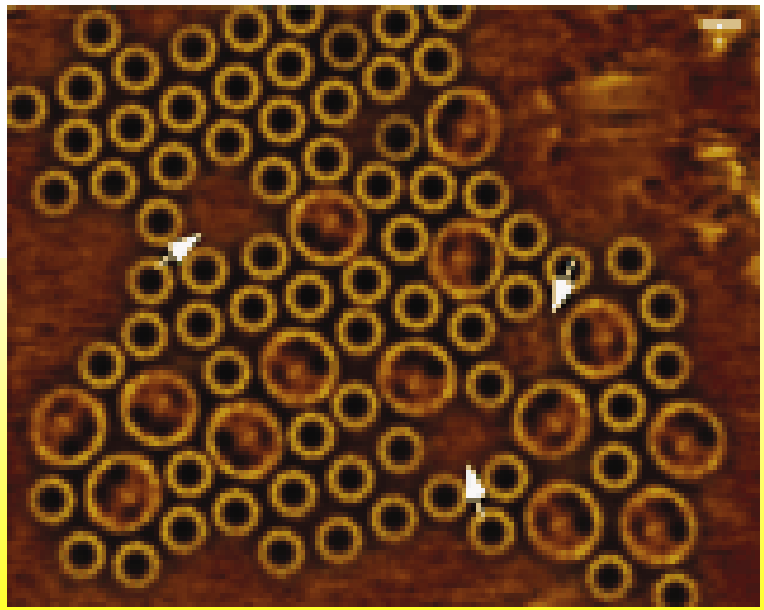


Photosynthetic system of purple bacteria in the intracytoplasmic membrane



System of pigments in RC


B800→B800
0.5ps
B800→B850
0.9ps
B850→B850
50-150fs
W
Q_B
L
M
H
LH2→LH1
2-4ps
LH1→RC
20-40ps



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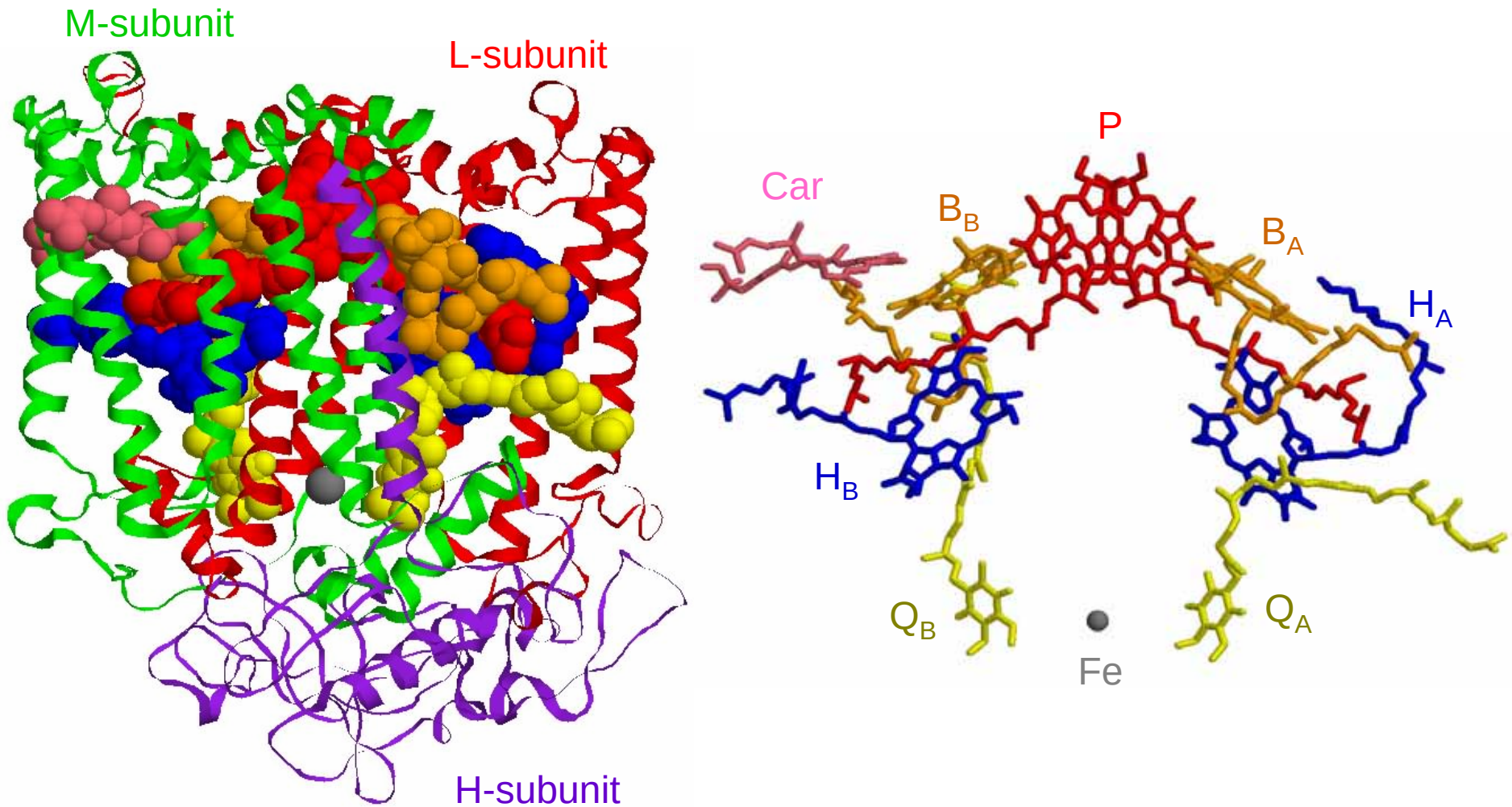
Structure of the photosynthetic RC



Shown above is the structure of RC from *Rhodobacter sphaeroides,*
Roszak, Hashimoto, Cogdell, and Frank, *et al.* (2004) *Structure,* **12**, 765.

Electron transfer in the RC

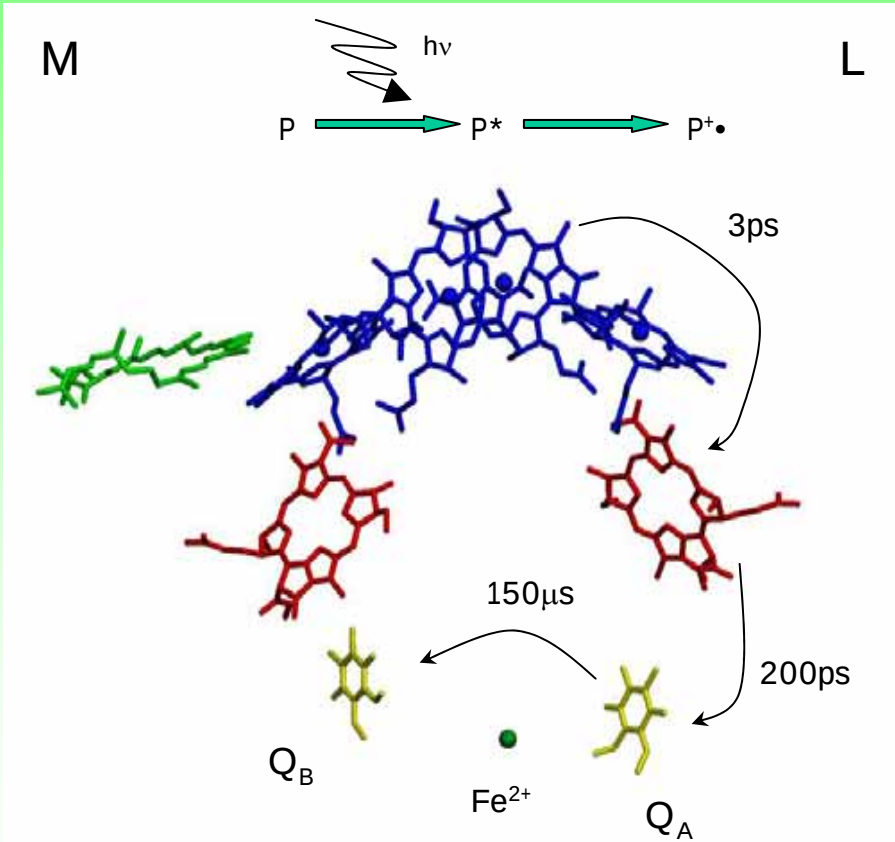
Bchl*a* 'Primary donor' P

Accessory Bchl*a*

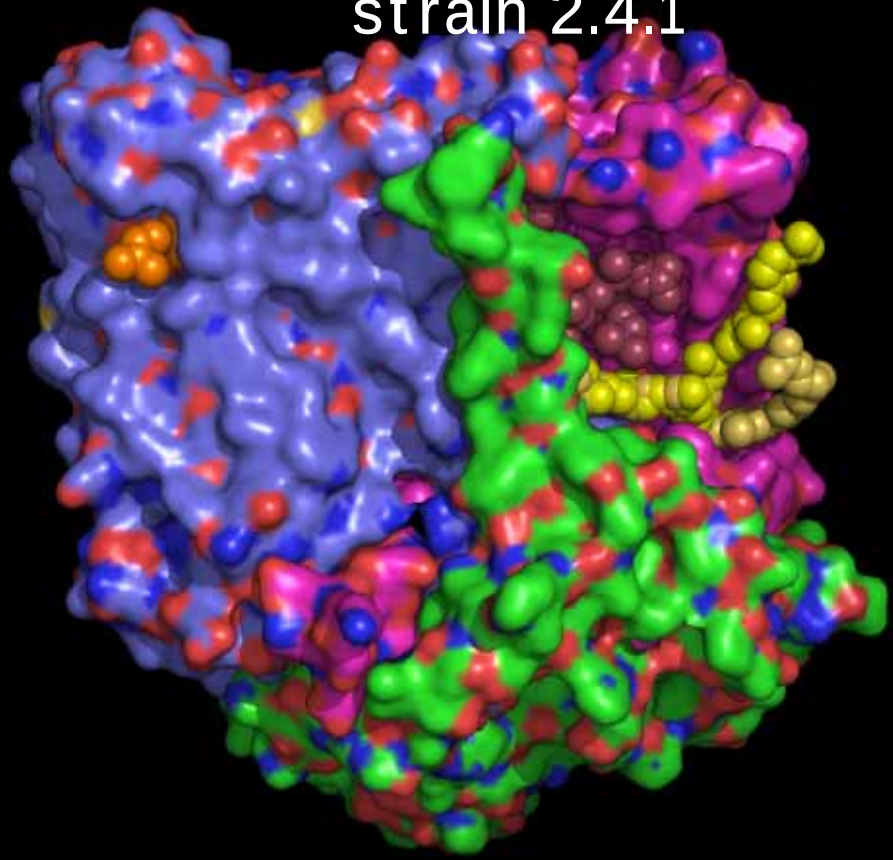
Pheophytin

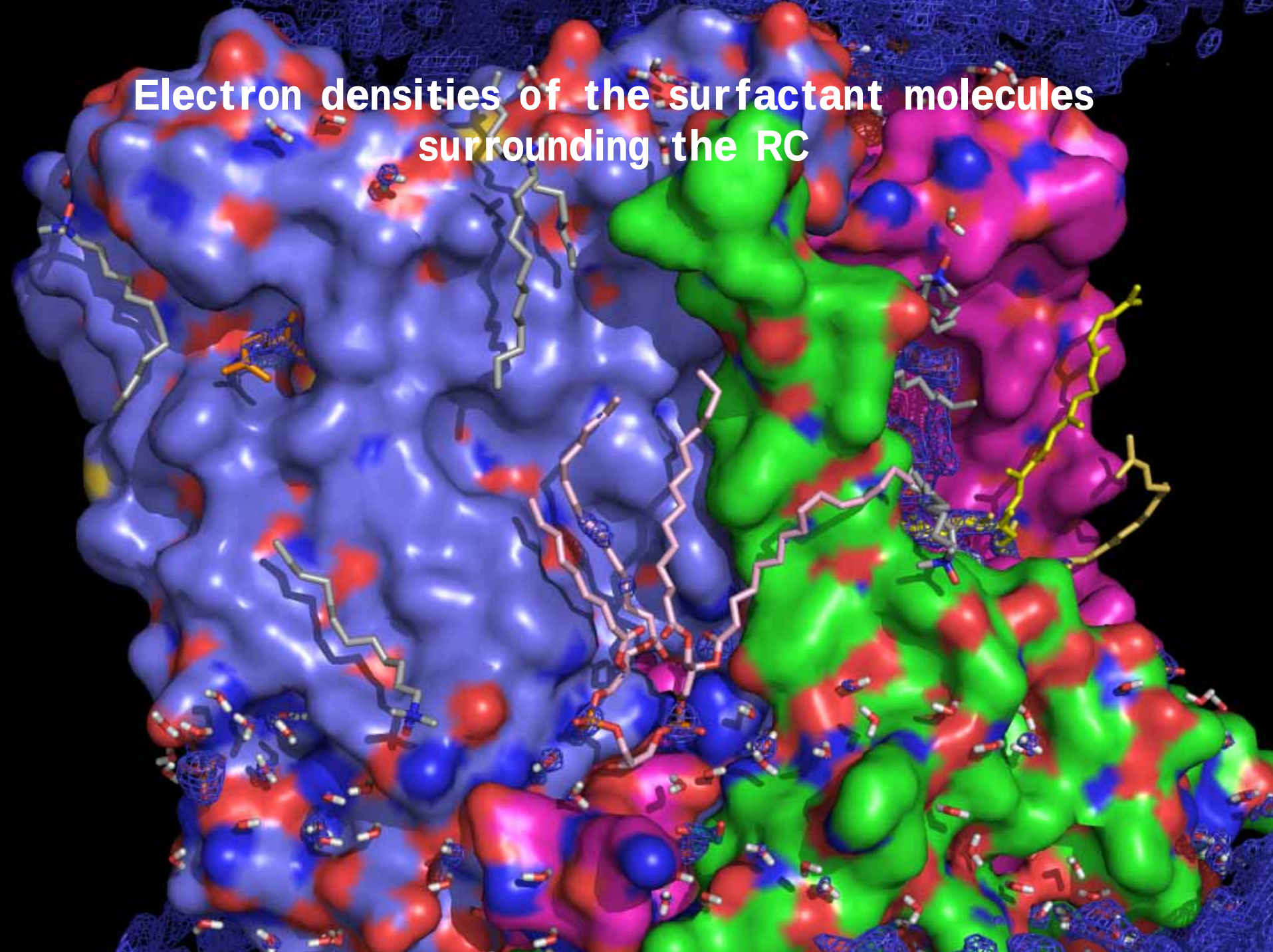
Spheroidene

Ubiquinone-10

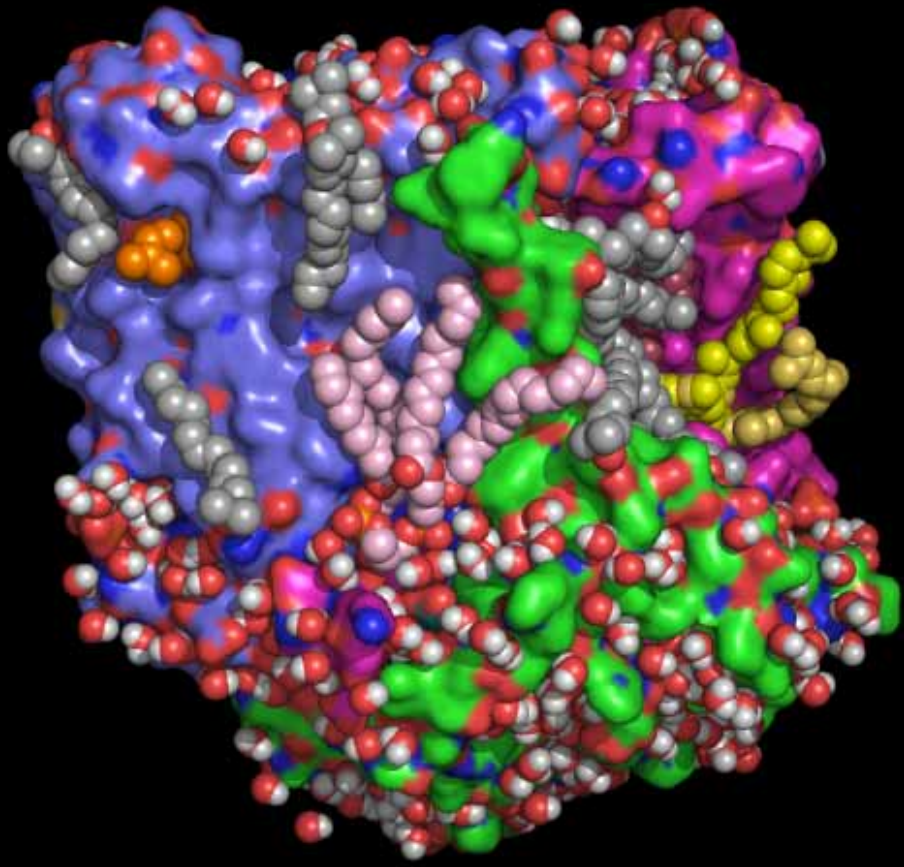


Our high-resolution (1.95 Å) X-ray crystal structure analysis of the wild-type RC from *Rba. sphaeroides* strain 2.4.1




Electron densities of the surfactant molecules
surrounding the RC

‘Solvent’ molecules surrounding the RC



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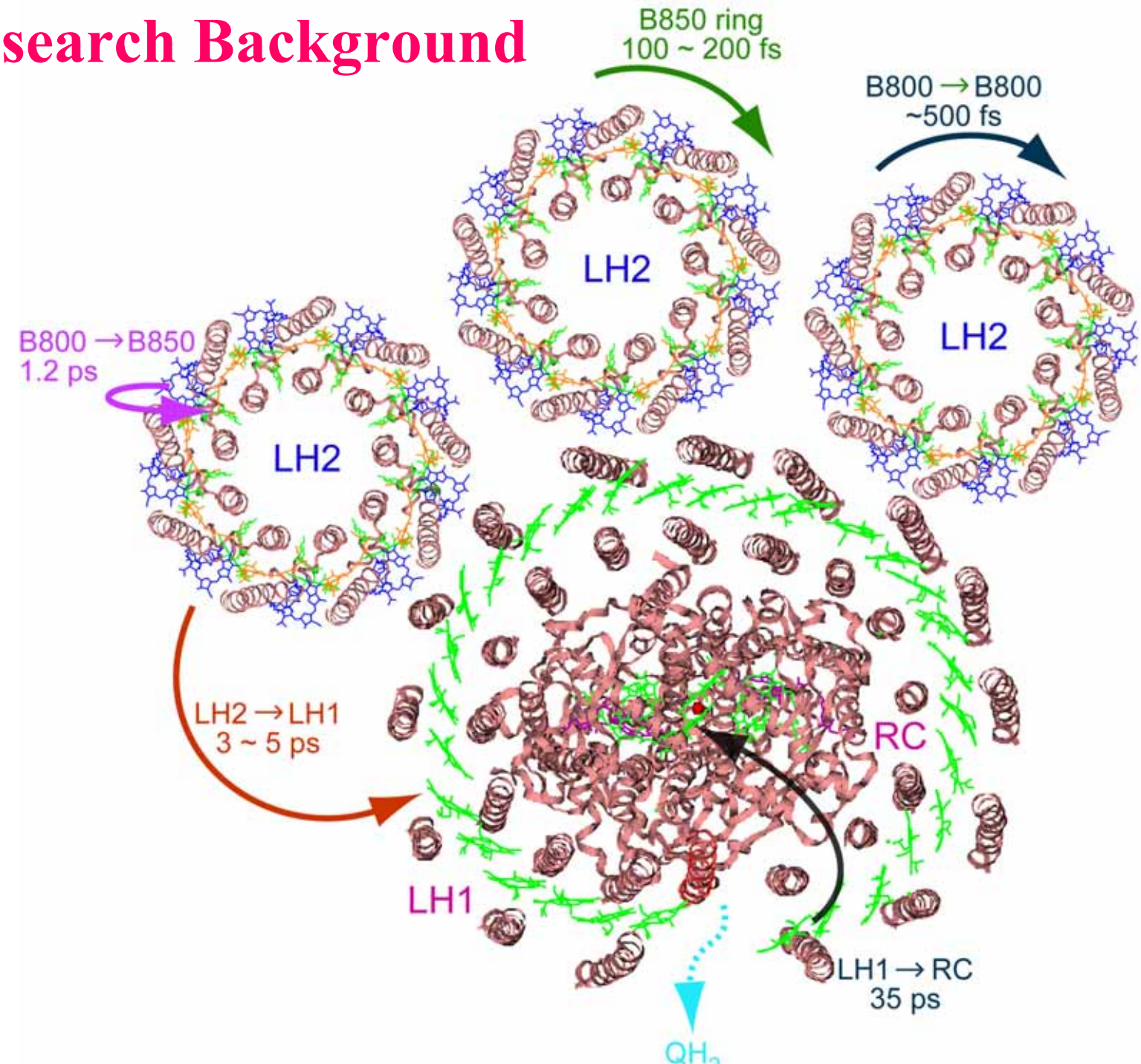
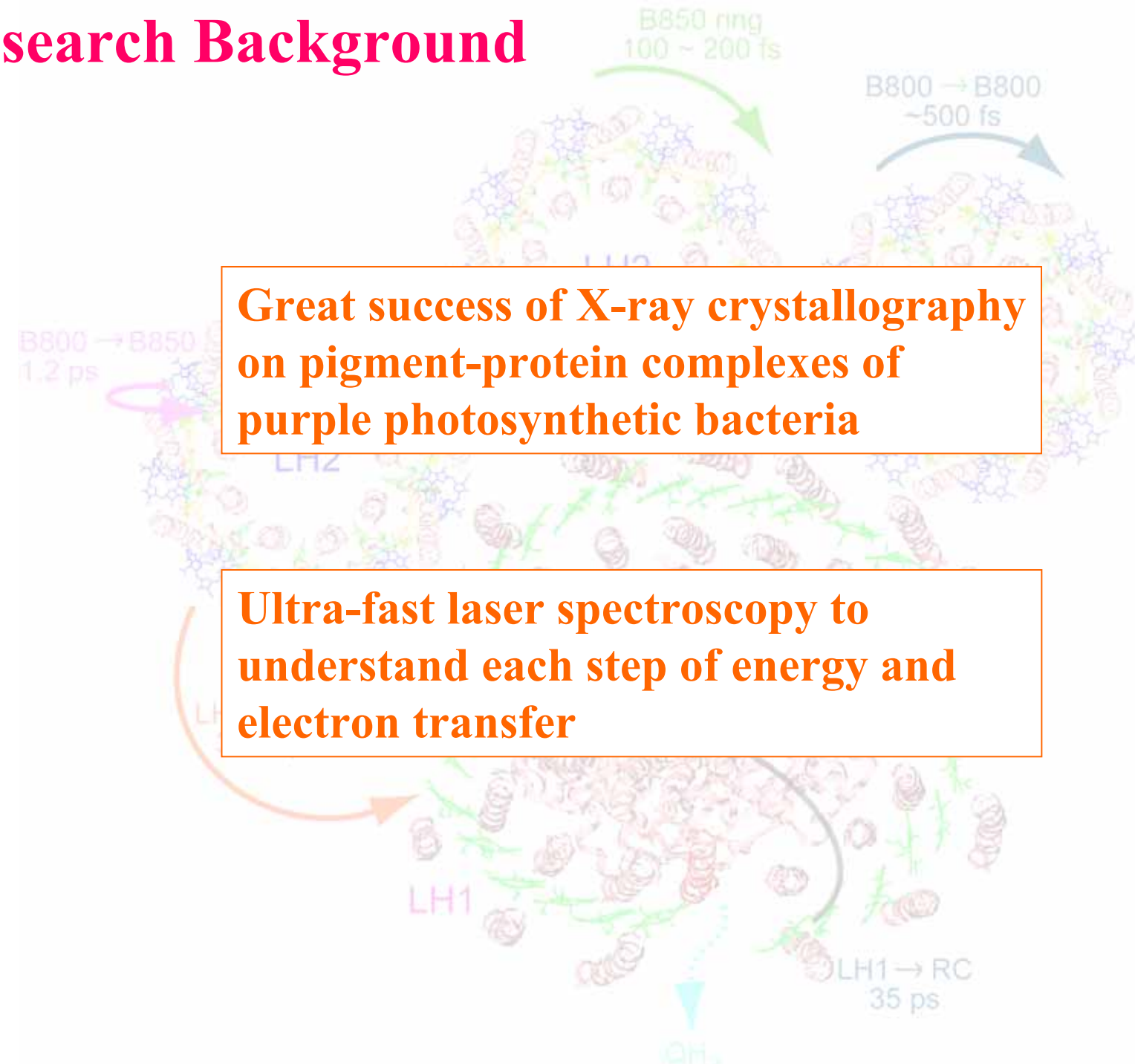
Research Background



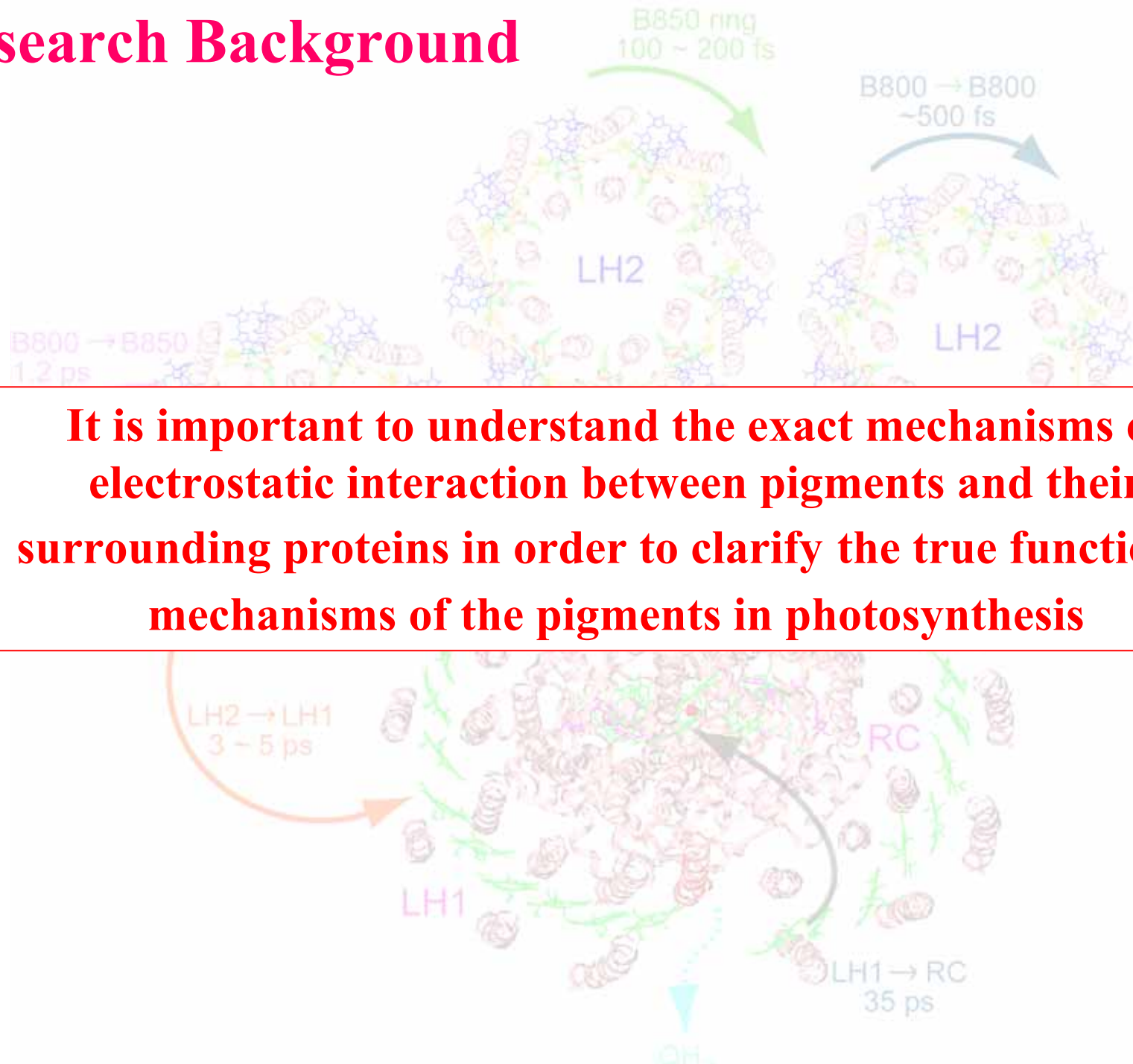
Research Background



Great success of X-ray crystallography on pigment-protein complexes of purple photosynthetic bacteria

Ultra-fast laser spectroscopy to understand each step of energy and electron transfer

Research Background

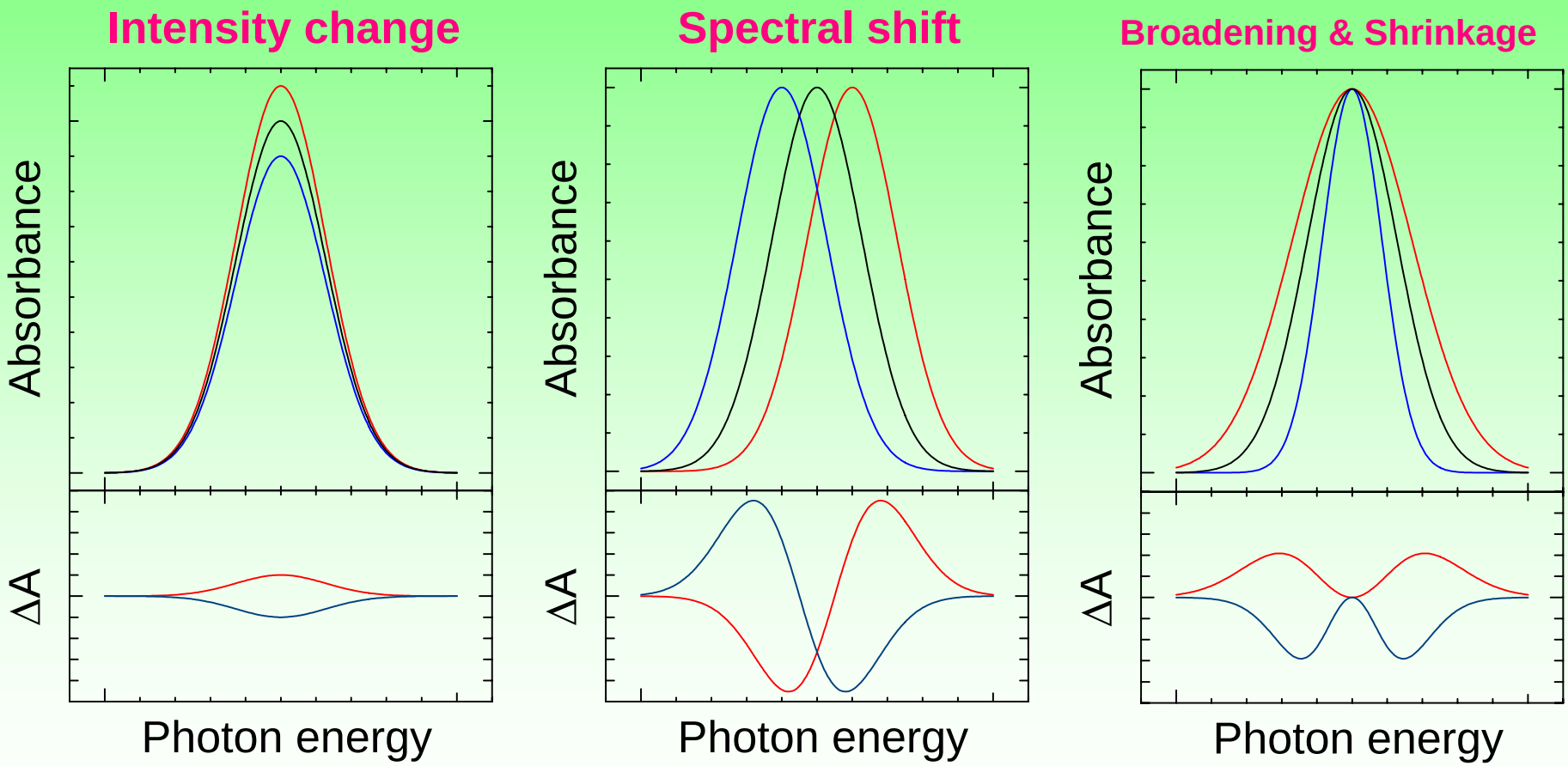


It is important to understand the exact mechanisms of electrostatic interaction between pigments and their surrounding proteins in order to clarify the true functional mechanisms of the pigments in photosynthesis

Characteristics of electroabsorption (Stark effect) spectroscopy

- Pigments in pigment-protein complexes are under the influence of internal electric field produced by the electrostatic potential of apoprotein
- Electroabsorption spectroscopy is one of the most promising methods to probe the pigment-protein interaction modulated by the external electric field

Optical absorption spectra under the influence of external or internal electric field



Liptay equation

$$\Delta A(\nu) = A(\nu, E_{\text{int}}) - A(\nu,0)$$

$$= (DA(\nu,0) + F\nu\frac{d(A/\nu)}{hd\nu} + H\nu\frac{d^2(A/\nu)}{2h^2d\nu^2})E_{\text{int}}^2 R(\chi,\zeta)$$

$$D = \frac{A^2}{M^2} + \frac{2B}{M},$$

$$F = \frac{2A\Delta\mu}{M} + \frac{\Delta\alpha}{2},$$

$$H = \Delta\mu^2$$

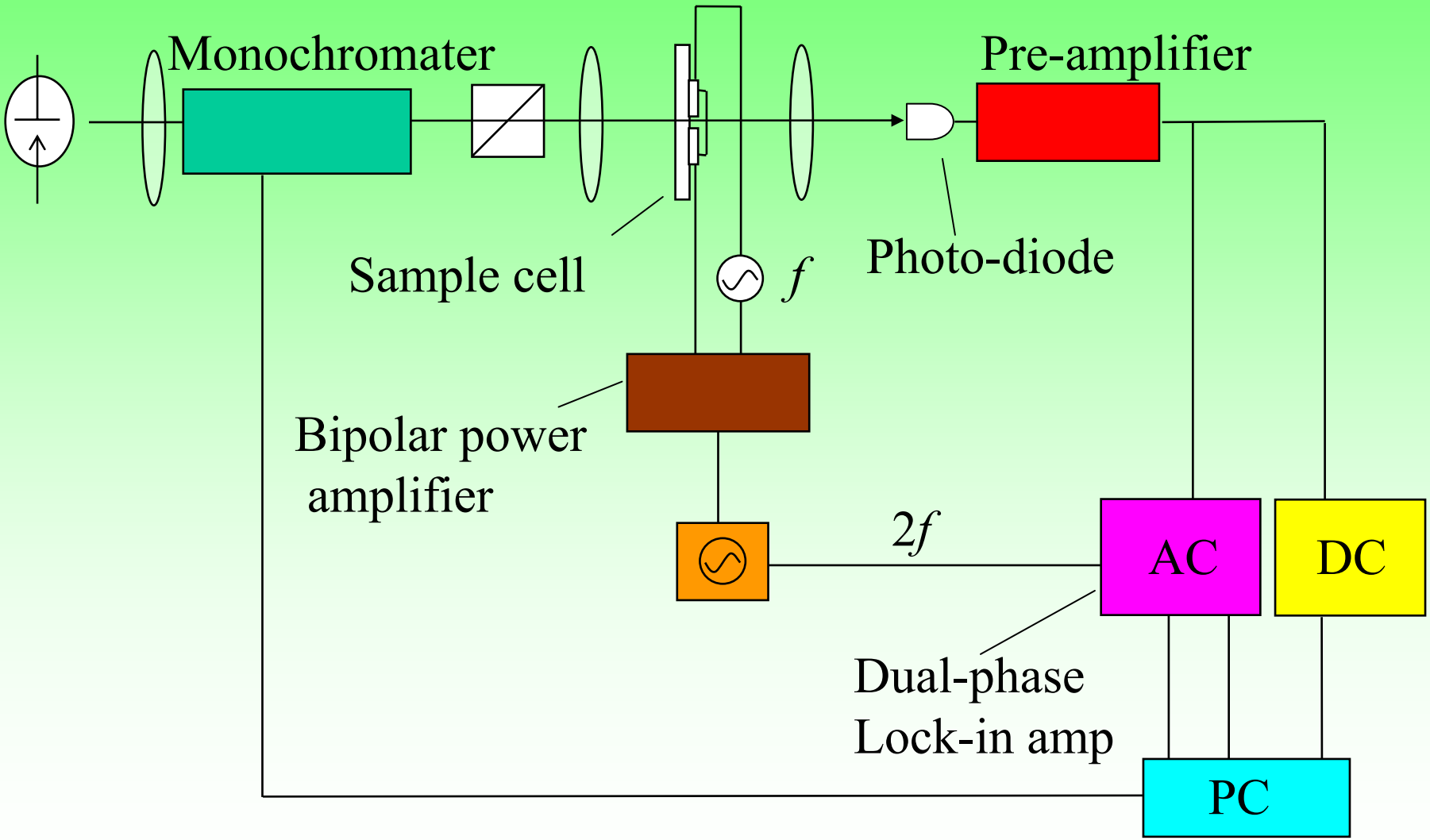
$$R(\chi,\zeta) = (5 + (3\cos^2\zeta - 1)(3\cos^2\chi - 1))/15$$

$$\mathbf{M}(F_{\text{int}}) = \mathbf{M} + \mathbf{A}\mathbf{E}_{\text{int}} + \mathbf{E}_{\text{int}}\mathbf{B}\mathbf{E}_{\text{int}}$$

$$h\nu_m(F_{\text{int}}) = h\nu_m(0) - \boldsymbol{\Delta\mu}\mathbf{E}_{\text{int}} - \frac{1}{2}\mathbf{E}_{\text{int}}\boldsymbol{\Delta\alpha}\mathbf{E}_{\text{int}}$$

← Stark effect

Experimental setup for electroabsorption spectroscopy



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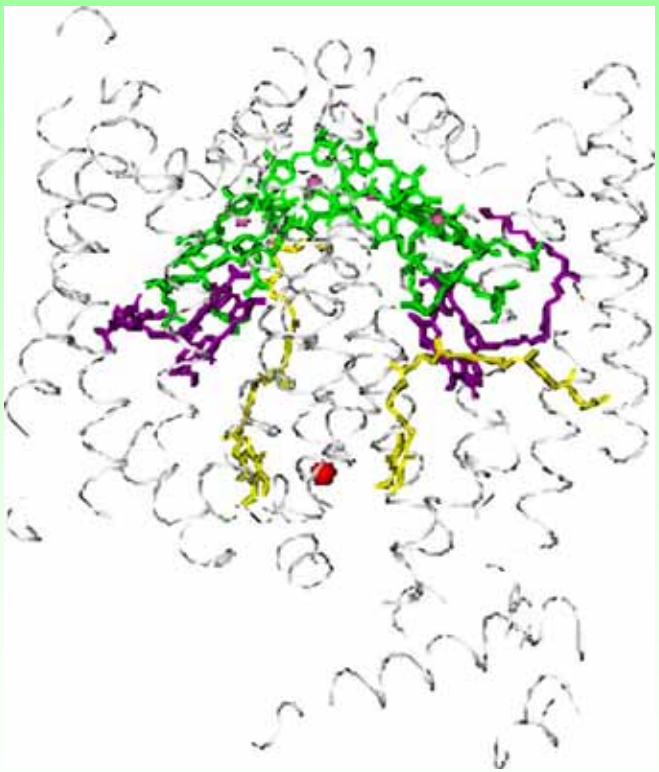
Objective

Evaluation of electrostatic effect of Car on Bchls (especially P) in RC

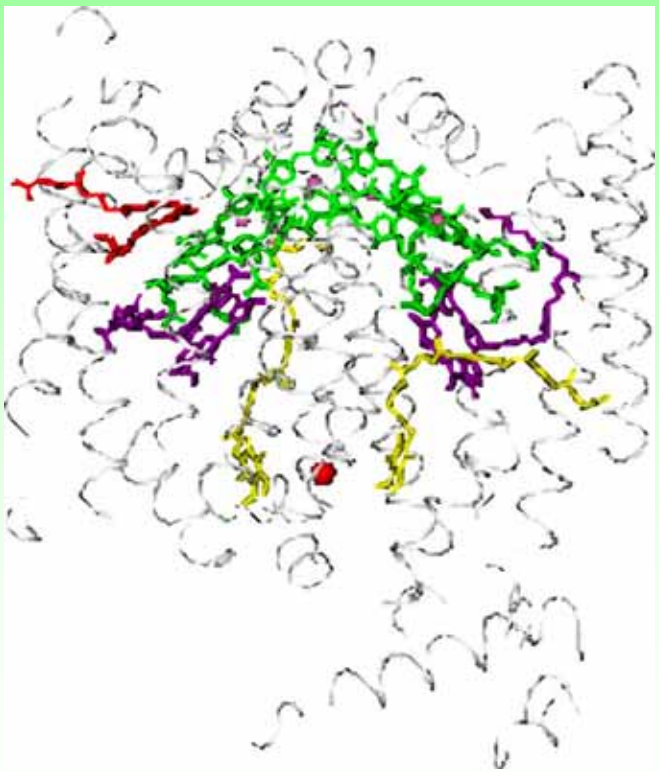


R26.1-RC

- **Electroabsorption (EA) spectra of two reaction centres were recorded**
- **One is prepared from *Rb. sphaeroides* strain R26.1 (*R26.1-RC*), which lacks carotenoid**

Objective

Evaluation of electrostatic effect of Car on Bchls (especially P) in RC

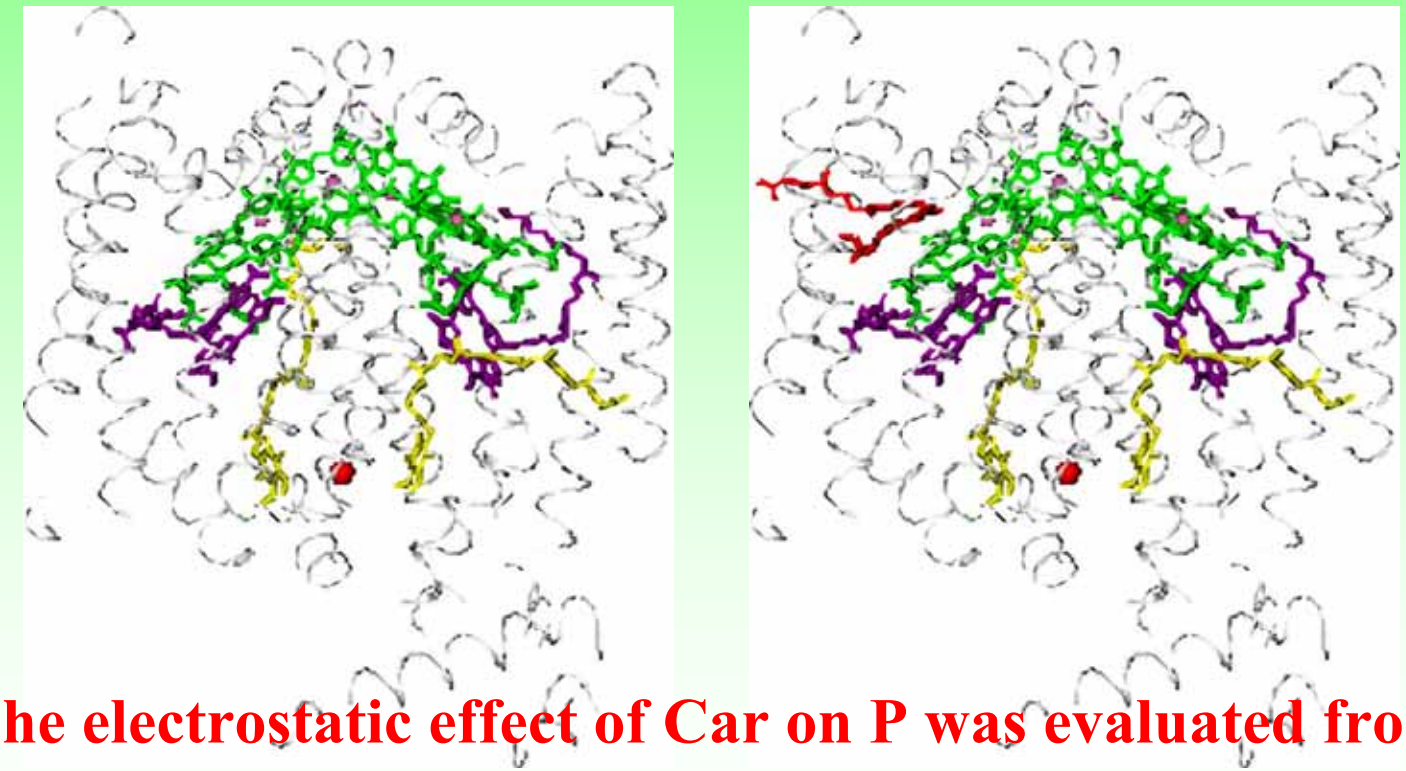


R26.1-RC+Car

- **Electroabsorption (EA) spectra of two reaction centres were recorded**
- **The other one is a reconstituted RC (*R26.1 -RC+ Car*) which was prepared by re-incorporating synthetic Car (3,4-dihydrospheroidene) into R26.1-RC**

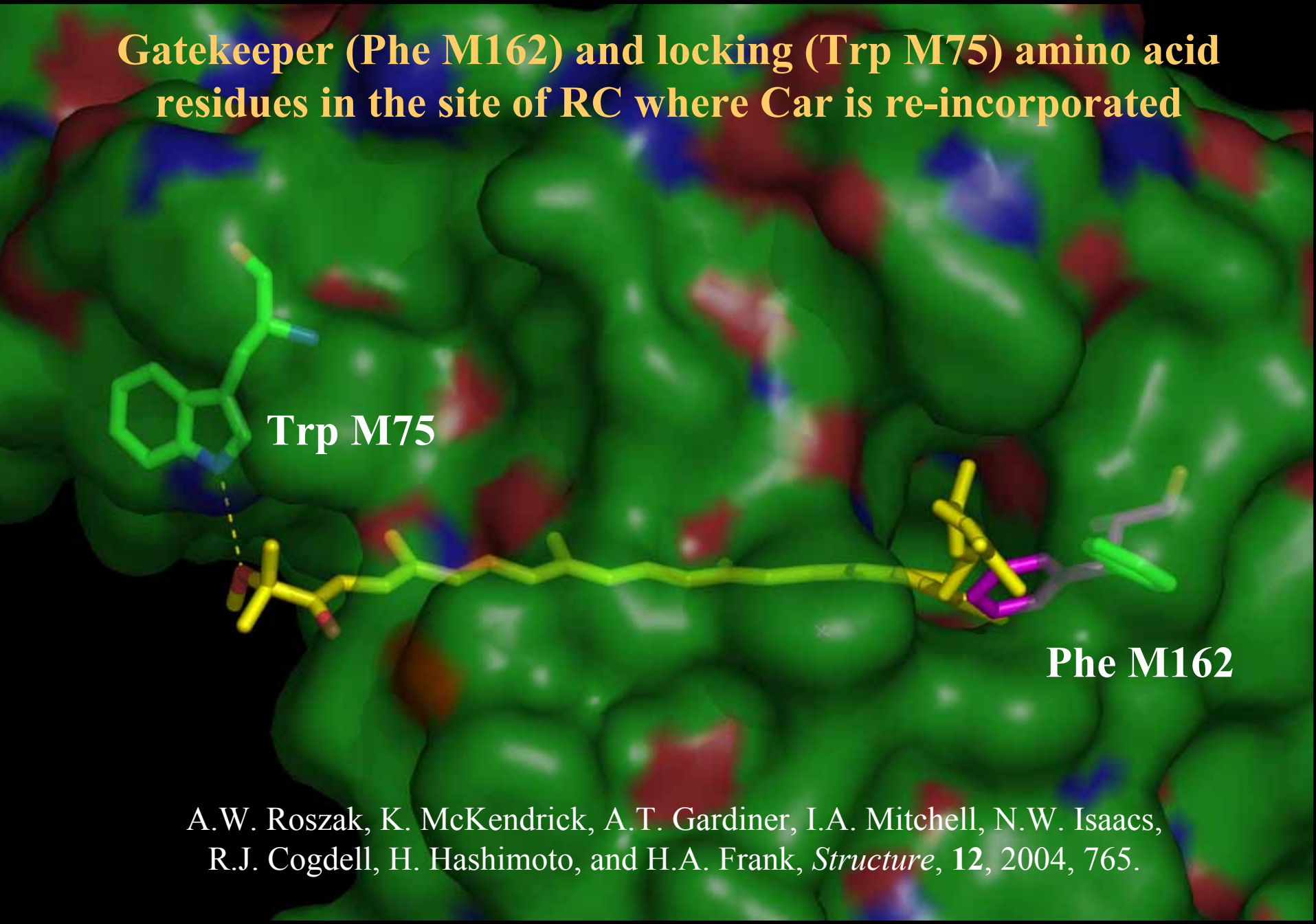
Objective

Evaluation of electrostatic effect of Car on Bchls (especially P) in RC



The electrostatic effect of Car on P was evaluated from the difference of these two spectra

Gatekeeper (Phe M162) and locking (Trp M75) amino acid residues in the site of RC where Car is re-incorporated



A.W. Roszak, K. McKendrick, A.T. Gardiner, I.A. Mitchell, N.W. Isaacs, R.J. Cogdell, H. Hashimoto, and H.A. Frank, *Structure*, **12**, 2004, 765.

Influence of static-electric field on the excited state of Bchls caused by the presence of Car

Electrostatic field F around Bchls in RC

$$\mathbf{F} = \mathbf{F}_a + \mathbf{F}_p + \mathbf{F}_c$$

F_a: applied electric field
F_p: pocket field
F_c: static-electric field due to Car

Absorption change due to applied electric field

Absorption change (ΔA) due to applied electric field can be written as,

$$\Delta A = \left(\frac{1}{3} DA + \frac{1}{6} F \frac{d\, A/\nu}{d\nu} + \frac{1}{6} \left|\Delta\boldsymbol{\mu}^*\right|^2 \frac{d^2\, A/\nu}{d\nu^2} \right) \left|\mathbf{F}_a\right|^2$$

$$D = \frac{1}{\left|\boldsymbol{\mu}_{\mathbf{eg}}\right|^2} \left(\mathbf{X}^2 + 2\boldsymbol{\mu}_{\mathbf{eg}} \mathbf{Y} \right) \approx 0 \qquad F = Tr\left[\Delta\boldsymbol{\alpha}\right] + 2 \frac{\boldsymbol{\mu}_{\mathrm{eg}} \mathbf{X}}{\left|\boldsymbol{\mu}_{\mathrm{eg}}\right|^2} \Delta\boldsymbol{\mu}^* \approx Tr\left[\Delta\boldsymbol{\alpha}\right]$$

$$\boxed{\Delta\boldsymbol{\mu}^* = \Delta\boldsymbol{\mu}_{\mathrm{int}} + \Delta\boldsymbol{\alpha} \cdot \mathbf{F}_{\mathrm{p}} + \Delta\boldsymbol{\alpha} \cdot \mathbf{F}_{\mathrm{c}}}$$

Influence of Car on Bchl

Change of Δμ value by static-electric field due to the presence of Car

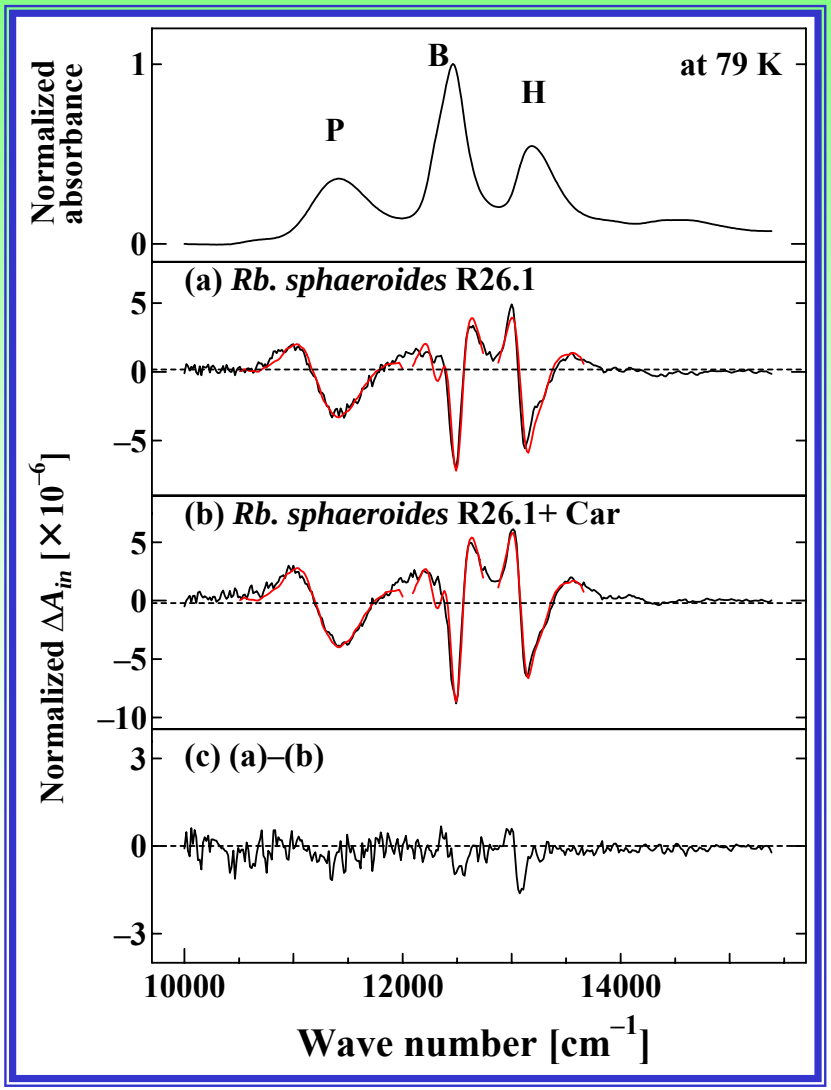
$$\Delta\mu_c = \Delta\alpha \cdot F_c$$

This change is expected to be observed by EA spectroscopy

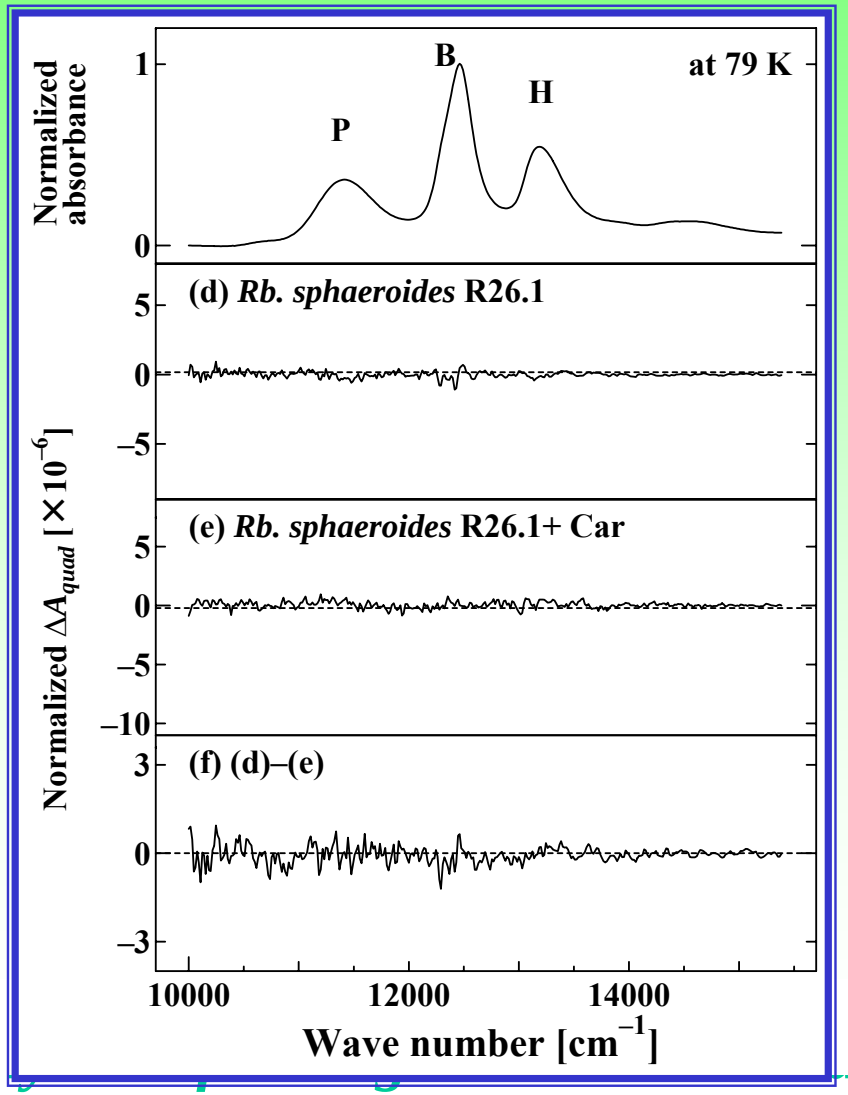
The contribution of Δμ is detected as the second-order derivative waveform of absorption spectra in EA spectra. Therefore, the second derivative waveform should be observed in the difference EA spectra between *R26.1-RC* and *R26.1-RC+Car*.

Electroabsorption spectra of RC's from *Rb. sphaeroides* R26.1 at 79 K

In-phase

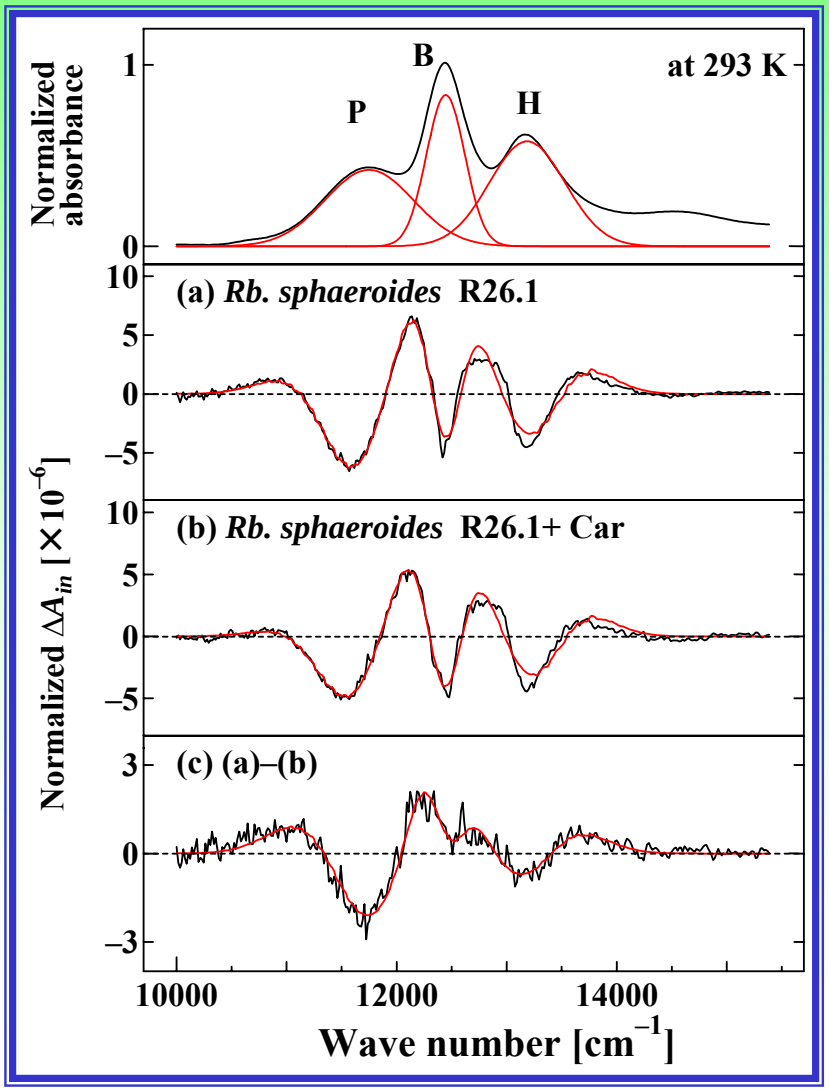


Quadrature-phase

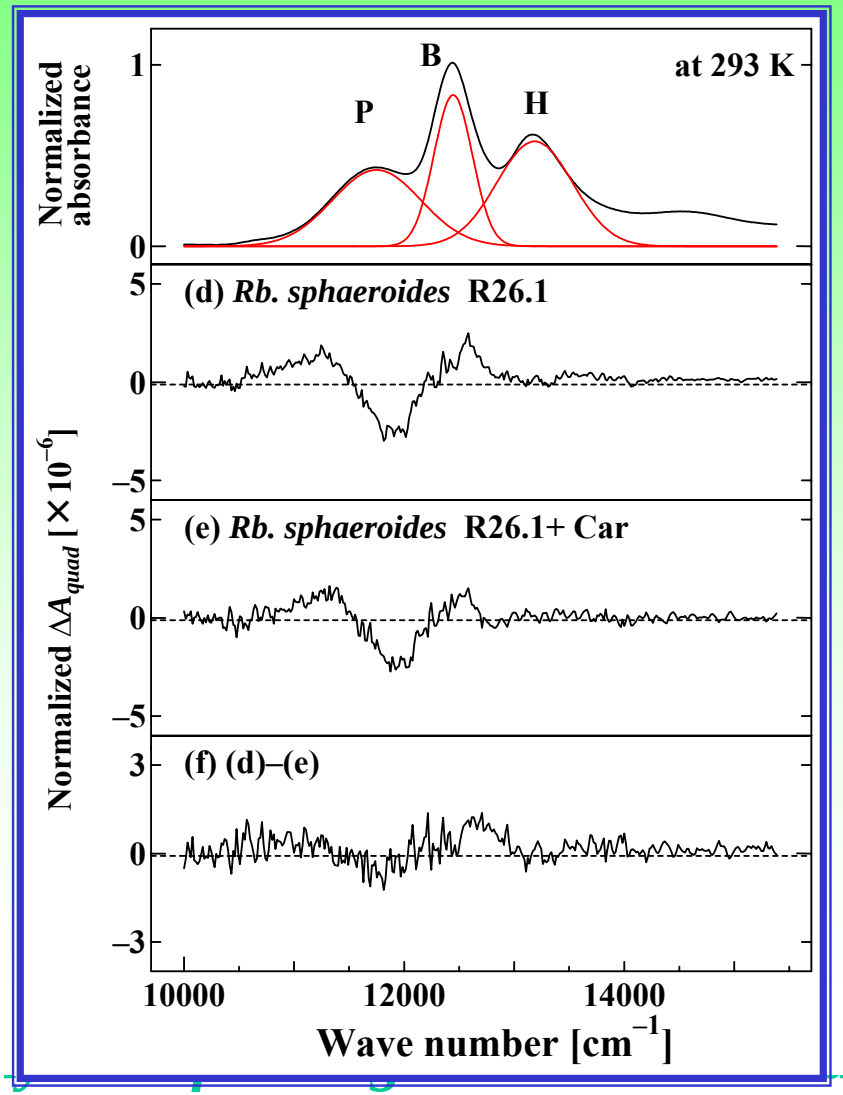


Electroabsorption spectra of RC's from *Rb. sphaeroides* R26.1 at 293 K

In-phase



Quadrature-phase



Nonlinear optical parameters of P band

	P band	D [10^{-18} $(m/f_L V)^2$]	Tr[$\Delta\alpha$] [$Å^3/f_L^2$]	$\Delta\mu^*$ [D/f_L]
79 K	R26.1-RC	-1.5 ± 0.4	$(2.2 \pm 0.3)\cdot 10^2$	6.5 ± 0.3
	R26.1+Car-RC	-1.8 ± 0.4	$(2.4 \pm 0.8)\cdot 10^2$	6.5 ± 0.3
293 K	R26.1-RC	3.9 ± 0.4	$-(1.7 \pm 0.1)\cdot 10^3$	**10.8 ± 0.4**
	R26.1+Car-RC	3.6 ± 0.4	$-(1.7 \pm 0.4)\cdot 10^3$	**9.0 ± 0.4**

$$\mathbf{F}_a = f_L \cdot \mathbf{F}_{ext}$$

F_{ext} is externally applied electric field.
f_L is a local field correction factor.

Even if the experimental errors are taken into account, the difference of $\Delta\mu^*$ between *R26.1-RC* and *R26.1-RC+Car* at 293 K is estimated to be ~1.0 [D].

Nonlinear optical parameters of B band

	B band	D [10^{-18} (m/f_LV)^2]	Tr[$\Delta\alpha$] [Å^3/f_L^2]	$\Delta\mu^*$ [D/f_L]
79 K	R26.1-RC	0.9 ± 0.2	-(1.2 ± 0.4)·10^2	2.8 ± 0.3
	R26.1+Car-RC	1.4 ± 0.5	-(1.1 ± 0.2)·10^2	2.9 ± 0.2
293 K	R26.1-RC	-7.1 ± 0.5	-(5.3 ± 3.6)·10	**2.1 ± 0.1**
	R26.1+Car-RC	-7.0 ± 1.7	-(5.8 ± 2.4)·10	**1.5 ± 0.2**

$\Delta\mu^*$ of B band is different between *R26.1-RC* and *R26.1-RC+Car* at 293 K.

Nonlinear optical parameters of H band

	H band	D [10^{-18} $(m/f_L V)^2$]	Tr[$\Delta\alpha$] [$Å^3/f_L^2$]	$\Delta\mu^*$ [D/f_L]
79 K	R26.1-RC	0.4 ± 0.2	-(8.4 ± 7.2)·10	4.4 ± 0.3
	R26.1+Car-RC	0.4 ± 0.1	-(0.9 ± 2.0)·10	4.3 ± 0.3
293 K	R26.1-RC	0.8 ± 0.2	(9.5 ± 2.3)·10	6.2 ± 0.4
	R26.1+Car-RC	0.8 ± 0.3	(10.0 ± 4.0)·10	5.7 ± 0.5

No significant difference is observed between R26.1-RC and R26.1+Car-RC in the nonlinear optical parameters of H band

Estimation of static-electric field induced by the presence of carotenoid

$$\Delta\mu_c = \Delta\alpha \cdot F_c \approx 1.0\,[D/f_L]$$

$$F_c \approx 1\times10^5\ [\mathrm{V/cm}] \qquad f_L = 2.2^{b}$$

Reported localized field around P is ~1✕10^6 [V/cm].[a]

The presence of Car causes **~10 % change** of the electrostatic environment around P. According to our results of X-ray crystallography of RC,[c] carotenoid binding pocket becomes smaller when Car is absent. This may cause some rearrangements of amino acid residues around P. As a consequence, the above ~10 % change should be occurred.

[a] T.R. Middendorf et al., BBA, **1143**, 1993, 223.
[b] M. Loche et. al., PNAS, **84,** 1987, 7537.
[c] A.W. Roszak et al., Structure, **12**, 2004, 765.

Conclusions on RC

- Carotenoid bound to the RC was found to affect on the electrostatic environment around P and B (B_B).
- The static electric field on P due to the presence of carotenoid was estimated to be ~ 1×10^5 [V/cm]. It corresponds to as high as 10% of the local electric field around P.
- Carotenoid could be one of the important factors that regulate the function of P (*vice versa*).

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Stark spectroscopy on the LH2 complex from *Rhodobacter sphaeroides* strain G1C; frequency and temperature dependence
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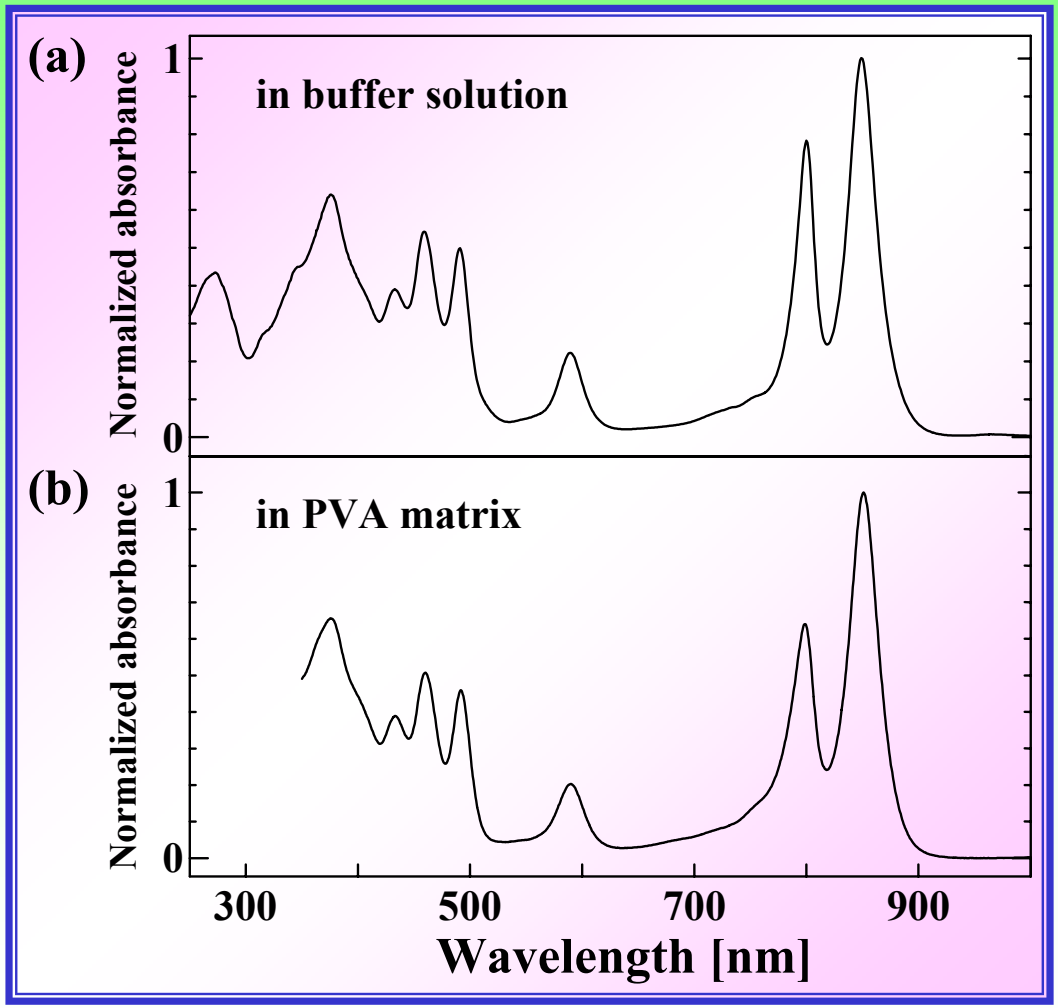
How can we evaluate electrostatic interaction between pigments and apoproteins?

- Most previous reports on the use of Stark spectroscopy to study photosynthetic pigment-protein complexes have only used *in-phase* signals
- More detailed information on dynamic electrostatic interactions with the environment can be obtained from *out-of-phase (quadrature-phase)* signal

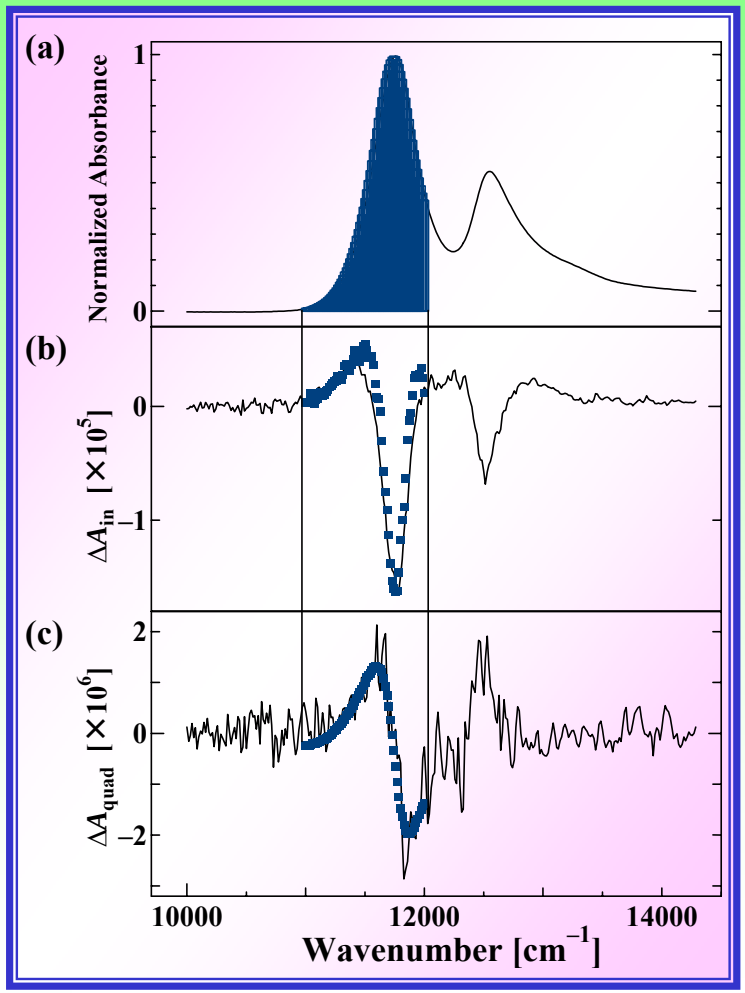
How can we evaluate electrostatic interaction between pigments and apoproteins?

By studying the frequency and temperature dependence of the quadrature-phase signals the nature of the pigment-protein interaction can be investigated!

Absorption spectra of the LH2 complex from *Rb. sphaeroides* G1C



Electro-absorption spectra of the LH2 complex from *Rb. sphaeroides* G1C



← in phase

← quadrature phase

Theoretical model to generate the phase-retarded signals

$$\Delta A = \frac{(\Delta\boldsymbol{\mu}^* \cdot \mathbf{E})}{h}\frac{\partial A}{\partial \nu} + \frac{1}{2}\frac{\mathbf{E} : \Delta\boldsymbol{\alpha} : \mathbf{E}}{h}\frac{\partial A}{\partial \nu} + \frac{1}{2}\frac{(\Delta\boldsymbol{\mu}^* \cdot \mathbf{E})^2}{h^2}\frac{\partial^2 A}{\partial \nu^2}$$

$$\Delta\boldsymbol{\mu}^* = \Delta\boldsymbol{\mu}_{\mathbf{mol}} + \Delta\boldsymbol{\alpha} : \mathbf{E}_{\mathbf{pocket}}$$

$$\mathbf{E}_{\mathbf{pocket}}(\mathbf{x}) = \mathbf{E}_{\mathbf{pocket}}(\mathbf{0}) + \mathbf{C} \cdot \mathbf{x}$$

$\Delta\mu^*$ is dipole-moment change upon photoexcitation
$\Delta\alpha$ is polarizability change upon photoexcitation

Dependence of the phase-retarded signals on the modulation frequency of the applied electric field

$$\frac{d^2\mathbf{x}}{dt^2} = -\gamma\frac{d\mathbf{x}}{dt} - \omega_0^2\mathbf{x} + \frac{q\cdot\mathbf{E}}{m}$$

$$\boxed{\mathbf{E}(t) = f_L\cdot\mathbf{E}_{ext}\sin(\omega t)}$$

$$\mathbf{x}(t) = \frac{q}{m}\frac{f_L\cdot\mathbf{E}_{ext}}{\left(\left(\omega_0^2-\omega^2\right)^2+\gamma^2\omega^2\right)^{1/2}}\sin(\omega t+\phi_p)$$

$$\tan\phi_p = \frac{\gamma\omega}{\omega^2-\omega_0^2}$$

Absorption change modulated with 2ω frequency

$$\Delta A_{2\omega} = \frac{x_0 C}{3h}\Delta\alpha\cdot\nu\frac{\partial A/\nu}{\partial\nu}\frac{f_L^2 E_{ext}{}^2\cos(2\omega t-\phi)}{2}$$
$$+\frac{1}{6}\left(\frac{\Delta\alpha}{h}\nu\frac{\partial A/\nu}{\partial\nu}+\frac{\Delta\mu^{*2}}{h^2}\nu\frac{\partial^2 A/\nu}{\partial\nu^2}\right)\frac{f_L^2 E_{ext}{}^2\cos(2\omega t)}{2}$$

In-phase signals detected by a dual-phase lock-in amplifier

$$\Delta A_{\text{in}} = \frac{1}{6}\left[\left(\frac{\Delta\alpha}{h} + \frac{2\mathrm{x}_0 \cdot C \cdot \Delta\alpha}{h}\cos\phi\right)\cdot\nu\frac{\partial A/\nu}{\partial\nu} + \left(\Delta\mu^*\right)^2\nu\frac{\partial^2 A/\nu}{h^2\partial\nu^2}\right]\cdot\frac{f_{\text{L}}^2\cdot E_{\text{ext}}^2}{2}$$

$$= \frac{1}{6}\left(F_{\text{in}}\cdot\nu\frac{\partial A/\nu}{h\partial\nu} + \left(\Delta\mu^*\right)^2\nu\frac{\partial^2 A/\nu}{h^2\partial\nu^2}\right)\cdot\frac{f_{\text{L}}^2\cdot E_{\text{ext}}^2}{2}$$

$$F_{in} = \Delta\alpha\cdot\left(1+2\mathrm{x}_0\cdot C\cos\phi\right) = \Delta\alpha\cdot\left(1+2\frac{qC}{m}\frac{\left(\omega_0^2-\omega^2\right)}{\left(\omega_0^2-\omega^2\right)^2+\gamma^2\omega^2}\right)$$

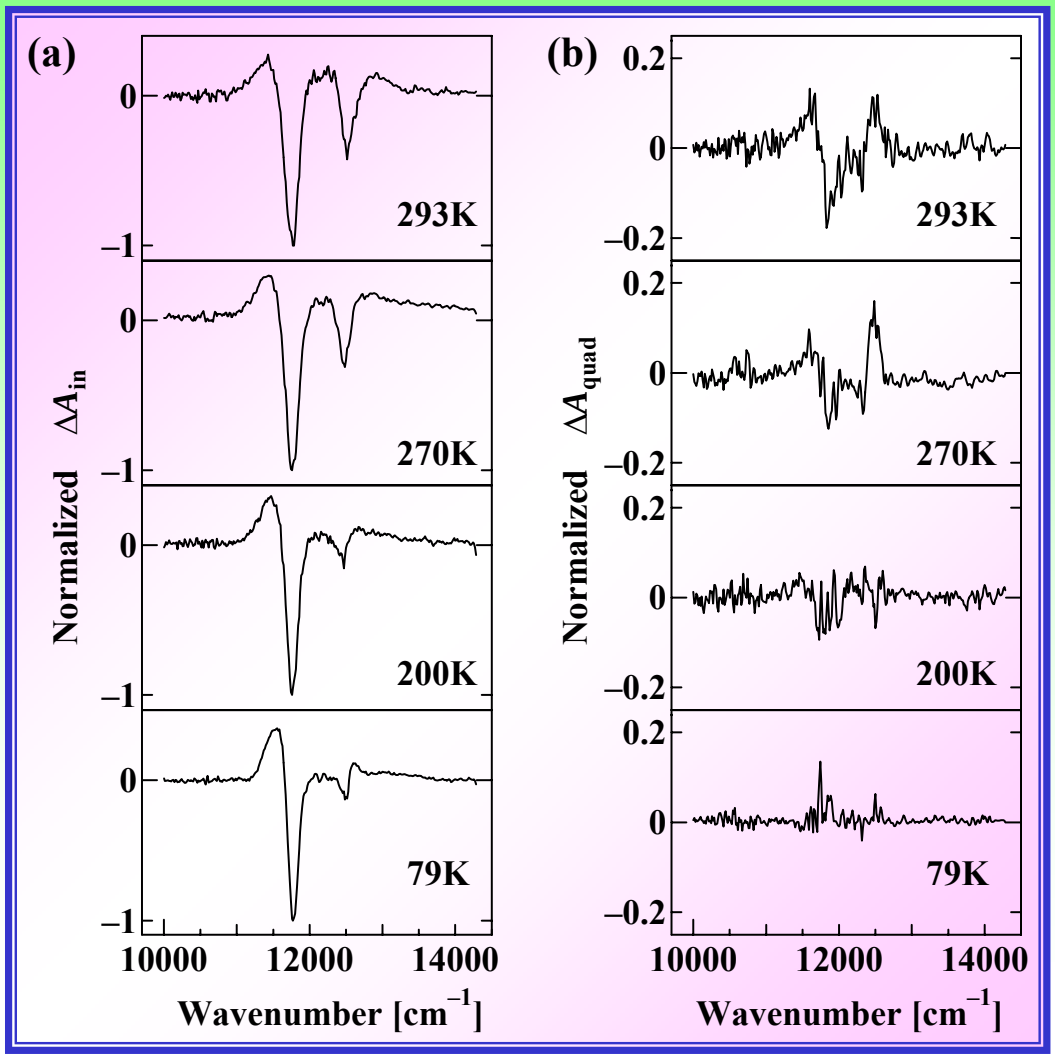
Quadrature-phase signals detected by a dual-phase lock-in amplifier

$$\Delta A_{\mathrm{quad}} = \frac{1}{6}\left(2\mathrm{x}_0 \cdot C \cdot \Delta\alpha \sin\phi\right) \cdot \nu \frac{\partial A/\nu}{h\partial\nu} \cdot \frac{f_{\mathrm{L}}^2 \cdot E_{\mathrm{ext}}^2}{2}$$

$$= \frac{1}{6} F_{\mathrm{quad}} \cdot \nu \frac{\partial A/\nu}{h\partial\nu} \cdot \frac{f_{\mathrm{L}}^2 \cdot E_{\mathrm{ext}}^2}{2}$$

$$F_{\mathrm{quad}} = 2\mathrm{x}_0 \cdot C \cdot \Delta\alpha \sin\phi = 2\Delta\alpha \frac{qC}{m} \frac{\gamma\omega}{\left(\omega_0^2 - \omega^2\right)^2 + \gamma^2\omega^2}$$

Temperature dependence of in-phase and quadrature-phase EA spectra



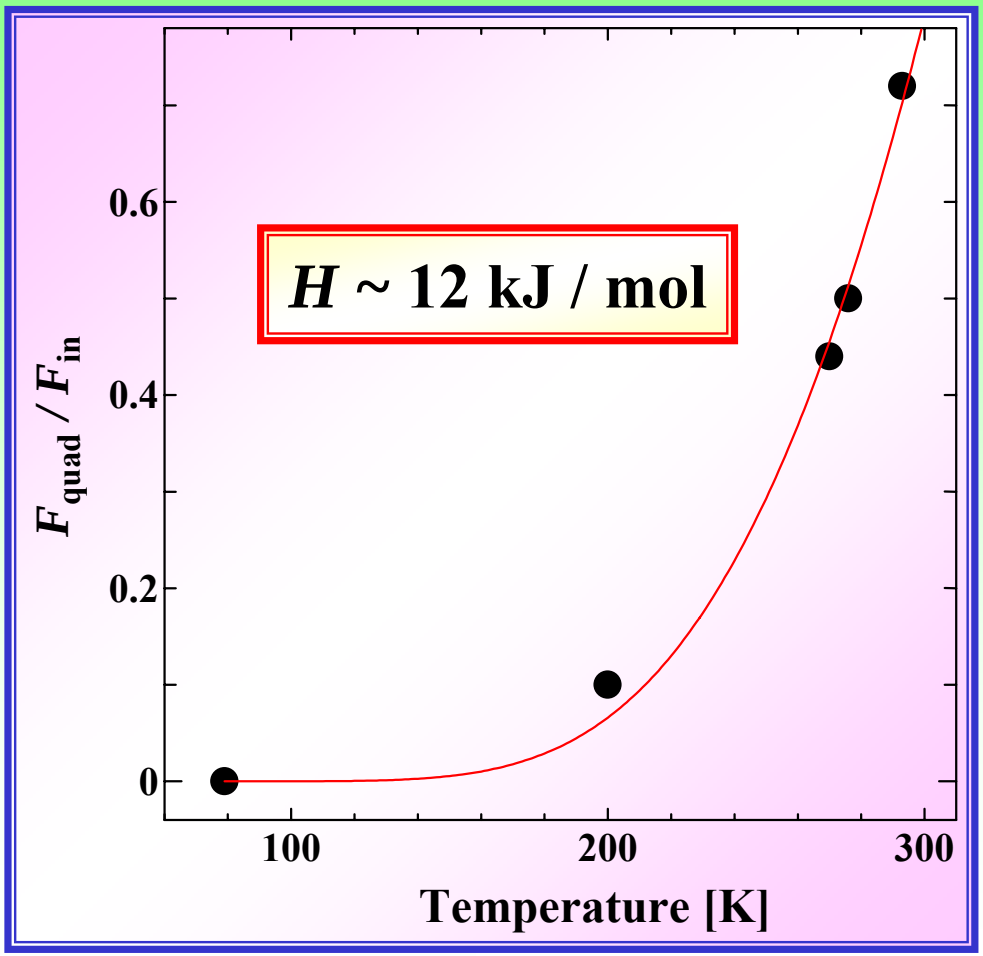
Temperature dependence of F_{quad} / F_{in} value

$$\frac{F_{quad}}{F_{in}} = \gamma \frac{\omega}{\frac{1}{2}\frac{m}{q \cdot C} \cdot [(\omega_0^2 - \omega^2)^2 + \gamma^2 \omega^2] + \omega_0^2 - \omega^2}$$

$$\propto \frac{1}{\tau_r} \propto \exp\left(-\frac{H}{RT}\right)$$

H is the activation energy that characterizes the relaxation factor γ

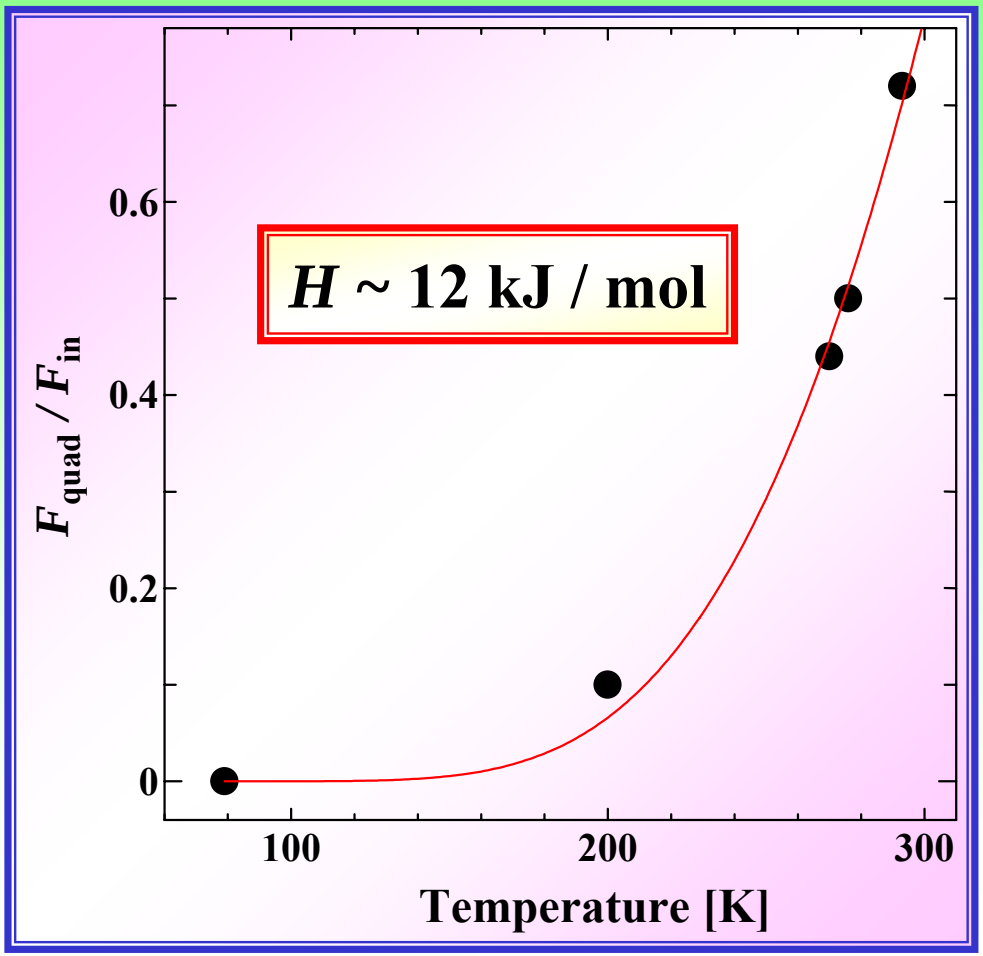
Temperature dependence of F_{quad} / F_{in} value



- Quite a number of molecular motions can be plausible candidates that generate the relaxation factor, γ.

  For example, local mode relaxation, crankshaft and kink motion, rotation of methyl group, side chain motion, local intermolecular rearrangements etc.

Temperature dependence of F_{quad} / F_{in} value



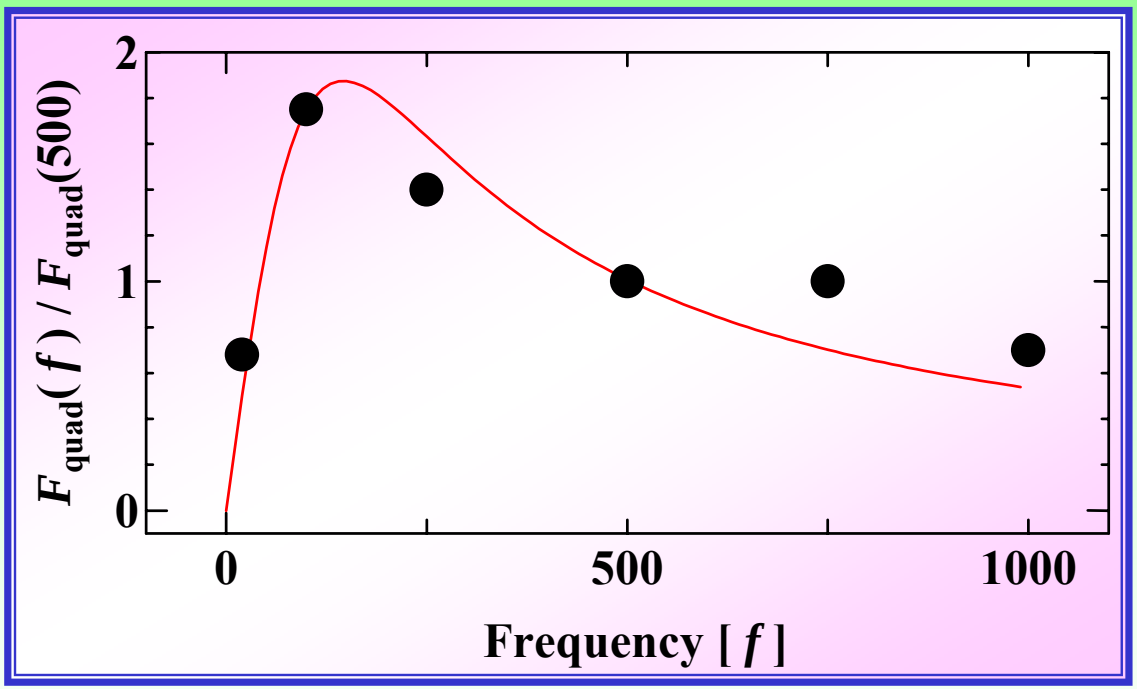
- Inspection of X-ray crystal structure of the LH2 complex from *Rps. acidophila* strain 10050, suggests that a plausible candidate for the physical origin of γ could be **either a local twisting motion of the apoprotein main chain, or rotational motion of apoprotein side chains**

Frequency dependence of F_{quad} value

$$F_{\text{quad}}(f) = 2\Delta\alpha \frac{qC}{m} \frac{2\pi\gamma f}{\left(\omega_0^2 - 4\pi^2 f^2\right)^2 + 4\pi^2\gamma^2 f^2}$$

$$\omega = 2\pi f$$

Dependence of the phase-retarded signals on the modulation frequency



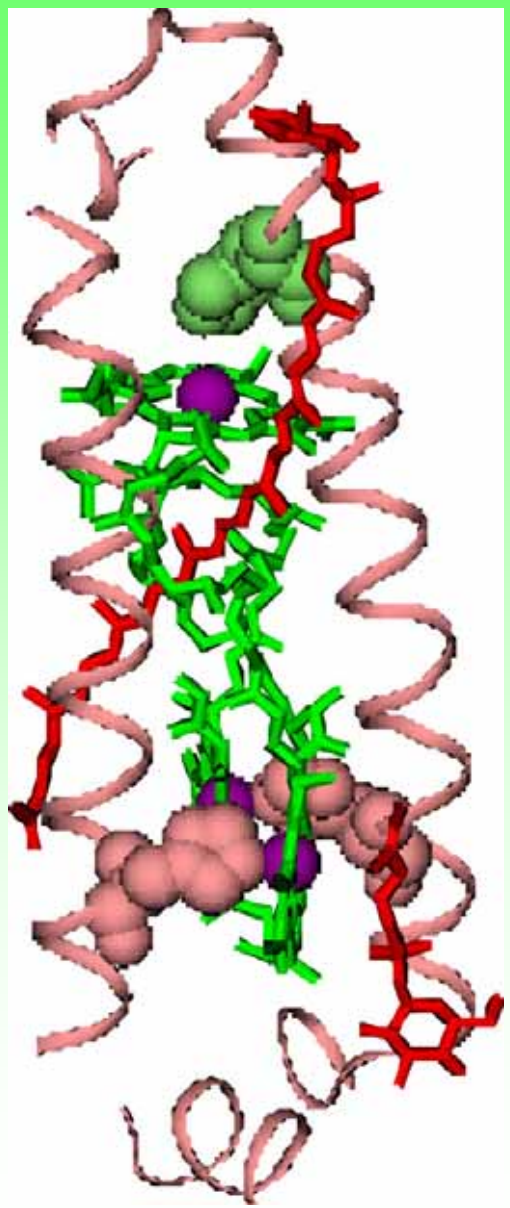
ω_0 ~ 11 kHz

sec
m sec
μ sec
n sec
p sec
f sec

Rotation of an aromatic side chain of an amino acid residue inside a protein

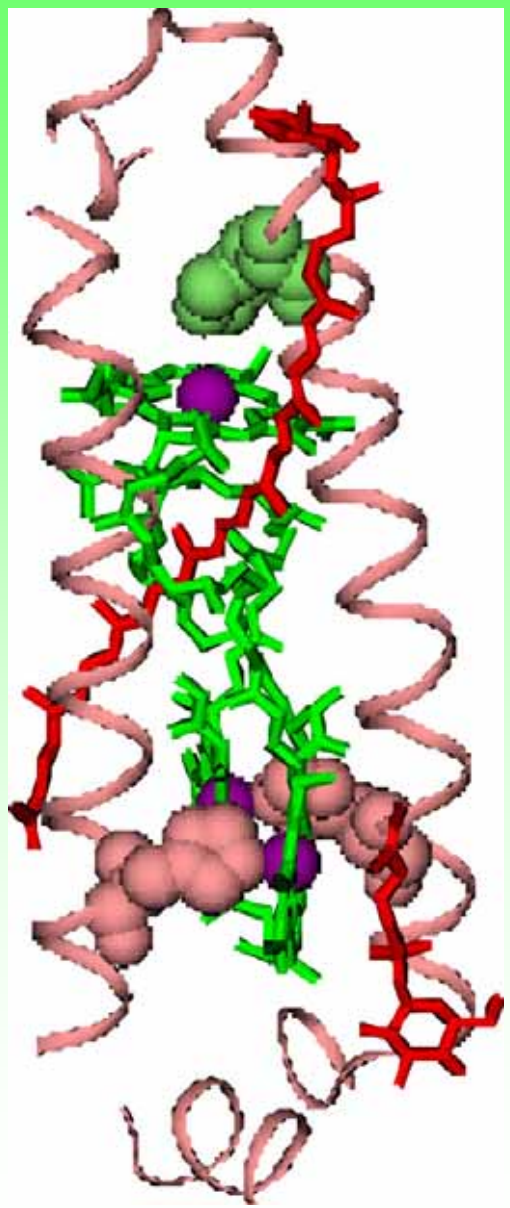
Deformation vibration

Stretching vibration



LH2 subunit of *Rps. acidophila* 10050

- The X-ray crystal structure of the LH2 complex from *Rps. acidophila* strain 10050 clearly shows that the Mg^{2+} ions at the centre of the B850 bacteriochlorin rings are liganded to histidine side chains.
- These histidine residues are prime candidates for the strong electrostatic interaction suggested by the presence of the phase-retarded signals.
- However, the resonance frequency of 11 kHz looks too low for a normal mode reflecting the Mg-His interaction (~1 THz).



The relevant normal mode could be an overall, coupled mode reflecting the total environment of pigment-protein interaction

LH2 subunit of *Rps. acidophila* 10050

Conclusions on LH2

- Electroabsorption (Stark effect) signals due to electrostatic interactions between the B850 pigments and surrounding apoproteins were detected as a phase-retarded signal using dual-phase lock-in detection
- The Arrhenius type activation energy (H = 12 kJ/mol) was determined from the temperature dependence of the phase-retarded signals. The resonance frequency (ω_0 = 11 kHz) was determined from their frequency dependence.
- The calculated thermodynamic and kinetic parameters can be accounted for by assuming a strong electrostatic interaction of the B850 pigments with the dynamic environment provided by the apoproteins.


Thank you for your attention !